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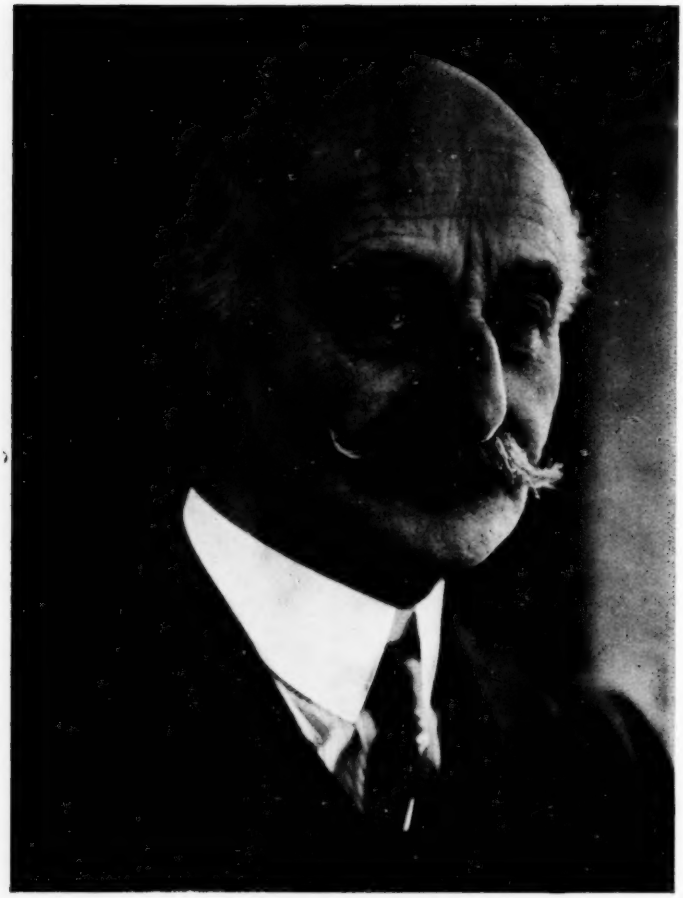

G. H. F. NUTTALL
In 1936

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GEORGE HENRY FALKINER NUTTALL
1862–1937

G. H. F. NUTTALL, Emeritus Professor of Biology in the University of Cambridge, died suddenly in London on 16 December 1937 at the age of 75. Nuttall was born in San Francisco, California, in 1862, the second son of Robert Kennedy Nuttall, M.D. (Aberdeen), and his wife, Magdalena, daughter of John Parrott, of San Francisco. Nuttall's father went first to Australia but later migrated to San Francisco where he practised till 1865. In that year the family returned to Europe, the children being educated in England, France, Germany and Switzerland. It was to this cosmopolitan upbringing that Nuttall owed his ability to speak several languages, an accomplishment which was to help him greatly in his work and travels and on the many occasions when he represented the University of Cambridge in foreign countries.

Nuttall returned to America in 1878 and, entering the University of California, obtained the M.D. degree in 1884.

In 1885 he joined the Johns Hopkins University in Baltimore, where he came under the influence of H. Newell Martin, who had been Huxley's demonstrator at the Royal College of Science, London. In 1886 Nuttall went to Germany, first to Breslau and later to Göttingen, where he remained for four years. While in Germany Nuttall was mainly interested in botany and zoology, and the study of these subjects led on to an interest in parasitology which was destined to become the main scientific interest of his life.

After travelling for a year, Nuttall returned to Baltimore in 1891 as assistant to W. H. Welch, Professor of Pathology. In 1893 he went to Europe, remaining in Germany till 1899; working part of the time at Göttingen and later in Berlin as voluntary assistant at the Hygienic Institute under Wolffhügel and Max Rubner. In 1895, while in Germany, he married Paula, daughter of Kammerherr von Oertzen-Kittendorf of Mecklenburg. In May 1899, at the invitation of the Regius Professor of Physic, Sir Clifford Allbutt, Nuttall came to Cambridge, and gave a course of lectures on bacteriology. A year later he was appointed University Lecturer in Bacteriology and Preventive Medicine. It was at this time that Nuttall founded the *Journal of Hygiene* which he edited up to the time of his death.

In 1904, in co-operation with Sir Patrick Manson, he persuaded the University of Cambridge to establish the first Diploma in Tropical Medicine and Hygiene. In 1906 Nuttall was appointed Reader in Hygiene and was elected a Fellow of Christ's College. He held the office of Reader for a few days only, for on 16 October 1906 he was elected the first Quick Professor of Biology and to the Professorial Fellowship of Magdalene College. According to the regulations of this Chair, until otherwise determined, the Professor should

devote himself to the "study of Protozoa, especially such as cause disease"; he should lecture only in one term on the results of research carried out in his laboratory or elsewhere, and should receive not more than £300 a year from the Quick Fund for "providing assistance and for the general maintenance of his laboratory". The regulation restricting his lectures brought his active teaching career to an end, and the last regulation hampered throughout his tenure of the professorship both his research and the expansion of his department by imposing the necessity for continual appeals for funds outside the University.

During the first years of his tenure of the Quick Professorship, Nuttall worked in a room under the medical museum which became known as the Quick Laboratory. In this uncomfortable room much valuable research was carried out by Nuttall and his co-workers. In 1908, Nuttall founded a second journal, *Parasitology*, and was its chief editor till 1933. In 1919, owing to the unsatisfactory conditions prevailing in the Quick Laboratory, Nuttall issued an appeal for funds with which to build an Institute for Parasitological Research. As a result of this appeal, a generous gift made by the late Mr and Mrs P. A. Molteno provided funds for the erection of the Institute which bears their name. In 1921, this building, the Molteno Institute for Research in Parasitology, was formally opened by Earl Buxton, and thus Nuttall realized an ambition for which he had striven for many years. In 1920 the "study of Parasitology" was substituted for the "study of Protozoa" in the regulation concerning the duty of the Quick Professor.

Nuttall resigned the Quick Professorship in 1931 and became Emeritus Professor of Biology. In 1932 he was presented by his colleagues and pupils with his portrait in oils by de Laszló.

During the period of his professorship he had raised considerable funds which were expended on the building, endowment, and research. He had formed large collections of parasites and a large and valuable library of books, journals, and reprints, all of which he presented to the Molteno Institute.

In spite of his multifarious activities, Nuttall found time to collect the photographs of many parasitologists and biologists, and this unique collection of more than 300 portraits now adorns the walls of the corridors in the Molteno Institute.

Nuttall's scientific work covered a very wide field; in addition to several books, he published papers in Bacteriology, Serology, Hygiene, Tropical Medicine and Parasitology.

Among his outstanding contributions to science was the discovery, made by him while working in Flügge's Institute (1888), that defibrinated blood possesses a strong bactericidal property against anthrax bacilli, and that this property disappears by heating the blood to 55° C. The results of this work were incorporated in a paper "A contribution to the study of immunity", which received the triennial Boylston Prize from Harvard University. This work founded the study of humoral immunity and was the forerunner of great

discoveries such as that of antitoxic immunity by workers like Behring and Kitasato. It occupied also a prominent place in the discussion of the question of humoral versus cellular immunity formulated by Metchnikoff.

In collaboration with Welch (1892), he studied in great detail the anaerobic gas-producing micro-organism known now as *Bacillus welchii*, the importance of which as a pathogenic agent was not fully appreciated until the Great War.

In collaboration with Thierfelder (1895–7) he carried out the first successful experiments on life under aseptic conditions. In these investigations, guinea-pigs, delivered by Caesarian section, were kept alive and fed in a specially devised chamber under bacteria-free conditions. These experiments solved one of the outstanding problems which preoccupied Pasteur and his pupil Duclaux. In fact, in a note to the Académie des Sciences, presented by Pasteur, Duclaux (1885) states that he failed to cultivate plants in soil rich in organic substances but devoid of micro-organisms. In an appendix to this note Pasteur remarks: "Sans vouloir rien affirmer, je ne cache pas que j'entreprendrais cette étude, si j'en avais le temps, avec la pensée préconçue que la vie dans ces conditions deviendrait impossible", and further "que le resultat soit positif et confirme la vue préconçue que je mets en avant, ou qu'il soit negatif et même en sens inverse, c'est-à-dire que la vie soit plus facile et plus active, il y aurait un grand intérêt à tenter l'expérience".

The fact that the results obtained by Nuttall and Thierfelder completely disproved the preconceived idea of Pasteur attracted the attention of biologists, and much work on this subject was subsequently carried out by the members of the Pasteur Institute of Paris. This work confirmed the results of Nuttall and Thierfelder and extended them to mammals, birds, Amphibia, insects, and plants. It was also clearly demonstrated that plants can easily be grown under aseptic conditions provided the mineral food is supplied to them and not complex organic substances as was done in Duclaux's experiments. In 1897, Nuttall turned his attention to the part played by arthropods in spread of disease, a subject to which he devoted the later period of his life. In 1899 he published his well-known paper, "On the rôle of insects, arachnids and myriopods, as carriers in the spread of bacterial and parasitic diseases of man and animals", which contains an exhaustive critical and historical review of the whole subject. Soon afterwards (1900–1) he became interested in the history of malaria in England, and, in collaboration with Cobbett, Strangeways, and others, undertook a survey of the distribution of *Anopheles* in England. He found that the three British species of these mosquitoes were to be found in all districts which were formerly malarious. The disappearance of malaria in England was therefore not due to the extinction of *Anopheles*. In collaboration with Shipley, he carried out an important investigation on the structure and biology of *Anopheles*, which is still regarded as a classic.

About the same time (1901) Nuttall turned his interest to the precipitin reactions. The production of precipitins in sera of animals inoculated with the clear filtrates of bacteria had already been demonstrated by Kraus (1897),

while Tchistovitch (1899), Bordet (1899), and Uhlenhuth (1900) had observed the formation of specific precipitation after inoculation of various bloods and sera. Nuttall soon confirmed these observations and greatly extended them.

The next three years he devoted to studying the application of the precipitin reaction to the phylogenetic relationships among the vertebrates. In 1904 he published his classical monograph, *Blood Immunity and Blood Relationship*, incorporating the results of an extensive investigation of precipitin reactions carried out on the blood of some six hundred species of animals. This investigation clearly demonstrated the existence of a distinct similarity in chemical structure of the blood in animals which are related phylogenetically. It threw a new light on the study of the phylogenetic relationships in the animal kingdom, and provided also a new method for the identification of minute traces of blood, a method which has had notable applications in forensic medicine.

After the publication of this monograph, Nuttall commenced his investigations on diseases transmitted to animals by ticks. By means of infected ticks (*Haemophysalis leachi*) sent to him by Lounsbury from South Africa, he succeeded in infecting dogs with piroplasmosis, a disease which is unknown in England. This was the first case of investigation of a disease imported by means of an infected vector. An important result of this investigation, which had great economic importance, was the discovery of the curative property of trypan blue for piroplasmosis in dogs, cattle, and sheep. This study was followed by an extensive investigation of the anatomy, biology, life history, and systematics of ticks, carried out in collaboration with Mr C. Warburton and Dr L. E. Robinson. The results of these investigations are incorporated in numerous papers and in the monographic study of ticks which occupied him on and off during the remaining years of his life. During this investigation Nuttall formed a very large collection of ticks from all parts of the world, probably the largest in existence, and rich in type specimens.

Most of his time during and after the Great War was given to the study of lice (*Pediculus*), their life history, biology, anatomy, and the problems of combating the spread of these parasites.

The published work of Nuttall is incorporated in almost 200 papers, the complete list of which is given later.

In his early life Nuttall had travelled in Mexico, Cuba, and North America, and it was probably during this period that he acquired the taste for Natural Science.

Nuttall's scientific work shows that he possessed to a remarkable degree the attributes of the acute observer, the naturalist, and the experimental scientist. These powers enabled him to recognize small specific characters with the intuition of the systematist and at the same time to unravel the most complicated anatomical structures. As the experimentalist he was able to devise complicated and well-controlled experiments and to overcome great technical difficulties in carrying them out.

As a naturalist he showed a real passion for collecting all sorts of specimens and in preserving, classifying, and cataloguing them. This work was always carried out with a great regard for permanence. He selected always the right type of containers or boxes for the specimens, unfading ink, and paper of lasting quality. The catalogues of his various collections have several hundreds of entries in his own handwriting giving the complete history of the specimens to which they refer. This passion for classification often spread beyond his scientific collections to the ordinary objects surrounding him. A pencil box, paper weight, or an ash tray on his desk or small gifts from his friends, however modest they were, had always some sort of history associated with them. They had real sentimental value to him and were classified as lovingly as specimens of scientific interest.

His scientific work was always marked by a great thoroughness and showed determination to cover the whole of the field connected with the problem under investigation. This thoroughness was to a great extent responsible for many of his achievements in Parasitology. On several occasions, however, it side-tracked him into work which required neither his great experience nor his mental ability and could easily have been left to his students.

Unfortunately, he often continued in these bypaths and persevered reluctantly but obstinately with what he used to call "clearing the deck", and this work kept him away from the study of more important general problems.

It is true that even in these wanderings, Nuttall made lasting contributions highly appreciated by other workers. Nevertheless, one wonders what other and greater discoveries he might have made had he persisted with the promising lines of work with which he started his scientific career.

Throughout his work, whether experimental or descriptive, Nuttall was always deeply interested in the history of the subject. His papers are marked by a thorough and first-hand knowledge of the literature and by exhaustive lists of references. He never grudged time or trouble in this task and spared no pains to obtain access to the most obscure paper or journal or the works of great naturalists of past centuries in order to acknowledge a contribution which had not been previously recognized.

On several occasions he collected and published exhaustive lists of literature, that on Ixodoidea, for example, containing more than 2000 titles. This difficult and exacting work brought him, however, some disappointment. He soon realized that while such lists help serious workers in their task, they unfortunately also encourage others who, while making extensive use of these compilations, fail to make the necessary acknowledgements.

In his editorial work in connexion with the publication of the *Journal of Hygiene* and *Parasitology*, he displayed the same thoroughness as in his research. His two journals became models upon which the publications of several scientific societies were based. He considered that the duty of an editor does not consist only in assuring the regular appearance of the journal or in keeping the work published up to a certain standard, but is also educa-

tional. In fact, much of his time and energy was devoted to the correction and improvement of papers and to correspondence with contributors. He advised and helped the young and inexperienced workers and in some cases re-wrote their papers.

As editor he exerted great influence on workers scattered all over the world, and this activity in a very great measure compensated him for the restriction in his teaching enforced by the regulations of the Quick Professorship.

Nuttall was of distinguished appearance and had a great charm of manner. He was an excellent raconteur and was fond of telling stories, full of incident and detail, of his early travels in Mexico, Cuba, and North America. He was invariably at ease in whatever company he found himself and was always capable of putting his companions at ease also. At international gatherings his knowledge of languages attracted to him numerous colleagues, and by his winning smile and his courteous remarks, couched in several languages if need be, he was able to weld together the members of an assembly, however heterogeneous, and to disperse the feeling of shyness and awkwardness so often apparent in such gatherings.

During his travels Nuttall had exceptional opportunities of establishing contact, usually developing into friendship, with many distinguished workers of international reputation. He became well acquainted with most of the great investigators in bacteriology, pathology, hygiene, parasitology, and tropical medicine, and he delighted to recount anecdotes illustrating their characters and their personal idosyncrasies.

He was, however, at his best discoursing with his class after the lecture or in the tea room of the Molteno Institute.

In addition to his scientific attainments, he read widely on a variety of subjects and could talk with real knowledge of such topics as heraldry and gardening. He was also an excellent draughtsman with a great interest in art. He was not exactly ambitious, but took great pleasure in the many marks of distinction conferred upon him. In 1904 he was elected a Fellow of the Royal Society, at the same time as his friend Shipley. He also received honorary degrees from the Universities of California, Strasburg, Egypt, South Africa, and Liège, and was elected corresponding member of many scientific societies. He received also the Belgian Order of Léopold II and the French Order of Commandeur de la Légion d'Honneur. He died suddenly, as he would have wished, still in the height of his activities and enthusiasm, on the eve of a dinner to be given by sixty colleagues in his honour on his retirement from the long and successful editorship of the *Journal of Hygiene*. His wife died in 1922, and he is survived by two sons and a daughter.

G. S. G.-S.
D. K.

LIST OF SCIENTIFIC PUBLICATIONS (1886–1938)[1]

By George H. F. Nuttall

1. Jastrow, J. & Nuttall, G. H. F. (vii. 1886). On the existence of a magnetic sense. *Amer. J. Psychic. Res.* **1**, 116–26. (Experimental research from the Psychophysical Laboratory of the Johns Hopkins University, Baltimore.)

2.† Nuttall, G. H. F. (vii. 1888). Experimente über die bakterienfeindlichen Einflüsse des thierischen Körpers. *Z. Hyg. InfektKr.* **4**, 353–94, 1 plate. (Demonstration of the bactericidal properties of the blood. Formed the basis of an essay "A contribution to the study of immunity", for which the author received the Boylston Prize from Harvard University.)

3.† Nuttall, G. H. F. (1890). *Beiträge zur Kenntniss der Immunität.* (Dissertation for the Degrees of M.A. and Ph.D., University of Göttingen.) 54 pp. Göttingen: L. Hofer.

4–5. Nuttall, G. H. F. (1891). A method for the estimation of the actual number of tubercle bacilli in tuberculous sputum. With a note on the general application of the method in bacteriology. *Johns Hopk. Hosp. Bull.* **2**, 67–76. *Also* published (1891) in German under the title of "Eine Methode zur Bestimmung der absoluten Anzahl der Tuberkelbacillen in tuberkulösem Sputum." *Z. klin. Med.* **21**, 23 pp.

6. Nuttall, G. H. F. & Wright, J. H. (1891). Report on the inoculation of thirteen guinea-pigs with the sputum of tuberculous cases undergoing the Koch treatment. *Johns Hopk. Hosp. Bull.* **2**, 77–9.

7. Nuttall, G. H. F. (1892). A chute for infected clothing intended for use in Hospitals. *Johns Hopk. Hosp. Bull.* **3**, 36, 2 figures. (The chute was subsequently adopted in Baltimore.)

8. Nuttall, G. H. F. (iv. 1892). The bacillus of influenza. *Science*, **19**, 193–4.

9. Nuttall, G. H. F. (1892). Einige Beiträge zur bakteriologischen Technik. *Zbl. Bakt.* **11**, 538–40, 2 figures.

10. Nuttall, G. H. F. (1892). Some medical notes made on a trip to Havana. *Johns Hopk. Hosp. Bull.* **3**, 44–5.

11. Nuttall, G. H. F. (1892). Tabulated results of the obstetrical practice of Robert Kennedy Nuttall, M.D., L.R.C.S., Ire. (deceased), covering a period of fifteen years. *Johns Hopk. Hosp. Bull.* **3**, 45.

12.† Welch, W. H. & Nuttall, G. H. F. (1892). A gas-producing bacillus (*Bacillus aerogenes capsulatus* n. sp.) capable of rapid development in the blood vessels after death. *Johns Hopk. Hosp. Bull.* **3**, 81–91.

13–14. Nuttall, G. H. F. (1893). *Hygienic Measures in relation to Infectious Diseases.* New York and London: G. F. Putnam's Sons. 101 pp. *Also* translated into German by Dr Otto Cahnheim under the title *Hygienische Maassregeln bei Infektionskrankheiten.* 80 pp. Berlin: Hirschwald.

15. Nuttall, G. H. F. (1895). Ueber den Einfluss von Schwankungen der relativen Feuchtigkeit der Luft auf die Wasserdampfabgabe der Haut. *Arch. Hyg., Berl.*, **23**, 184–92, 1 figure.

15*a*. Nuttall, G. H. F. (i. 1895). Bemerkung zu der Arbeit von Walliczeck, Ueber die baktericiden Eigenschaften. *Zbl. Bakt.* **17**, 131–2.

16. Nuttall, G. H. F. (1895). Ueber das Yunker'sche Kalorimeter und den Heizwerth des Berliner Leuchtgases. *Hyg. Rdsch.* **5**, 351–3, 1 figure.

17. Nuttall, G. H. F. (1895). Ein einfacher für Mikroskope verschiedener Konstruktion verwendbarer Thermostat. *Zbl. Bakt.* **18**, 330–2, 2 figures.

[1] The more important publications are marked †.

18. NUTTALL, G. H. F. (1895). Hygienic notes made on a short journey through Italy in 1894. (Report to the American Public Health Association as delegate to the International Medical Congress at Rome.) *J. Amer. publ. Hlth Ass.* **20**, 26–33, 2 figures.

19. NUTTALL, G. H. F. & THIERFELDER, H. (1895). Ueber thierisches Leben ohne Anwesenheit von Bakterien im Verdauungskanal. *Verh. physiol. Ges. Berl.* p. 37 (preliminary note).

20–22.† NUTTALL, G. H. F. & THIERFELDER, H. (1895). Thierisches Leben ohne Bakterien im Verdauungskanal. *Hoppe-Seyl. Z.* **21**, 109–21, 1 plate. (1896) Part II. *Hoppe-Seyl. Z.* **22**, 62–73. (1897) Part III. *Hoppe-Seyl. Z.* **23**, 231–5.

23–25.† NUTTALL, G. H. F. (1897). Zur Aufklärung der Rolle, welche Insekten bei der Verbreitung der Pest spielen. Ueber die Empfindlichkeit verschiedener Thiere für dieselbe. *Zbl. Bakt.* **22**, 87–97. *Also* appeared as translation into English in the *Publ. Hlth Rep., Wash.*, and *Vet. J.* 1897.

26. NUTTALL, G. H. F. (1897). Has adipocere been observed in India? *Indian med. Gaz.* **32**, no. 4.

27.† NUTTALL, G. H. F. (1898). Zur Aufklärung der Rolle, welche stechende Insekten bei der Verbreitung von Infektionskrankheiten spielen. Infektionsversuche an Mäusen mittels mit Milzbrand, Hühnercholera und Mäuseseptikämie inficirter Wanzen und Flöhe. *Zbl. Bakt.* **23**, 625–35.

28–29. NUTTALL, G. H. F. (1898). The first International Leprosy Conference, Berlin 1897. *Philad. med. J.* **1**, 59. *Also* translated into German: *Hyg. Rdsch.* **8**, 49–67.

30. NUTTALL, G. H. F. (1898). Neuere Untersuchungen über Malaria, Texasfieber und Tsetsefliegenkrankheit. *Hyg. Rdsch.* **8**, 1084–1103, 1137.

31. NUTTALL, G. H. F. (1899). The poisons given off by parasitic worms in man and animals. *Amer. Nat.* **33**, 247–9.

32.† NUTTALL, G. H. F. (1899). On the role of insects, arachnids and myriapods, as carriers in the spread of bacterial and parasitic diseases of man and animals. A critical and historical study. *Johns Hopk. Hosp. Rep.* **8**. Fasciculus of 154 pp., 2 plates. (Abstract in *Brit. med. J.* 9 Sept. 1899.)

33. NUTTALL, G. H. F. (1899). Die Rolle der Insekten, Arachniden und Myriapoden als Träger bei der Verbreitung von durch Bakterien und thierische Parasiten verursachten Krankheiten des Menschen und der Thiere. Eine kritisch-historische Studie. *Hyg. Rdsch.* **9**, 209–20, 275–89, 393–408, 503–20, 606–20 (translation of the above).

34. NUTTALL, G. H. F. (1899). La teoria zanzare malaria (translation of No. 35 by Dr Claudio Sforza). *G. med. Eserc.* fasc. no. 11, repr. 28 pp.

35. NUTTALL, G. H. F. (1899–1900). Die Mosquito-Malaria-Theorie. *Zbl. Bakt.* **25**, 162–70, 209–16, 245–7, 285–96, 337–46.

36. NUTTALL, G. H. F. (1899–1900). Neuere Forschungen über die Rolle der Mosquitos bei der Verbreitung der Malaria. Zusammenfassendes Referat. *Zbl. Bakt.* **26**, 140–7, and **27**, 193–6, 218–25, 260–4, 328–40 (exhaustive bibliography).

37. NUTTALL, G. H. F. (7. VII. 1900). [*Anopheles* and Malaria in England] Abstract of paper accompanying demonstration before the Pathol. Soc. of London. Cambridge Meeting, 30 June 1900. *Brit. med. J.* **2**, 20–1.

38. NUTTALL, G. H. F. (1900). *Rôle des Insectes, des Arachnides et des Myriapodes dans la transmission et la dissemination des maladies bactériennes et parasitaires de l'homme et des animaux.* Étude critique et historique. Traduit de l'anglais par le Dr Levrier, Médecin de 1re Classe des Colonies. 161 pp. Bordeaux: Imprimerie du Midi, P. Cassignol (translation of no. 32).

39. NUTTALL, G. H. F. (1900). The mosquito-malaria-theory. *J. trop. Med.* (*Hyg.*), **2**, 198–200, 231–3, 245–7, 305–7; **3**, 11–13, etc., 2 plates.

40. NUTTALL, G. H. F. (1900). Article "Filariasis", in *Encycl. Med.* **3**, 463–85, with two plates and bibliography.
41. NUTTALL, G. H. F. (1900). Article "Microorganisms", in *Encycl. Med.* **8**, 72–85.
42. NUTTALL, G. H. F. & STRANGEWAYS PIGG, T. (1900). Article "Glanders", in *Encycl. Med.* **4**, 158–71.
43–44. NUTTALL, G. H. F. (1900). An apparatus for making roll-cultures. *Philad. med. J.* 25 Aug., *also* (in German) *Zbl. Bakt.* **27**, 605–9, 2 figures.
45. NUTTALL, G. H. F. (IV. 1901). In Memoriam. Walter Myers. *J. Hyg., Camb.*, **1**, 285–7, with portrait.
46.† NUTTALL, G. H. F. & DINKELSPIEL, E. M. (11. V. 1901). Experiments upon the new specific test for blood. Preliminary note. *Brit. med. J.* **1**, 1141.
47. NUTTALL, G. H. F. (15. VI. 1901). Hibernation of *Anopheles* in England. *Brit. med. J.* **1**, 1473.
48.† NUTTALL, G. H. F. & DINKELSPIEL, E. M. (VII. 1901). On the formation of specific anti-bodies in the blood following upon treatment with the sera of different animals, together with their use in legal medicine. *J. Hyg., Camb.*, **1**, 367–87.
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50.† NUTTALL, G. H. F. (14. IX. 1901). The influence of colour upon *Anopheles*. *Brit. med. J.* **2**, 668–9.
51. NUTTALL, G. H. F. (XI. 1901). On the formation of specific antibodies in the blood, following upon treatment with the sera of different animals. *Amer. Nat.* **35**, 927–32.
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REPORT ON HELMINTHS COLLECTED FROM AN INDIAN CHUKAR, WITH DESCRIPTIONS OF TWO NEW SPECIES OF *RAILLIETINA* FUHRMANN, 1920

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From the Department of Zoology, University College of Wales, Aberystwyth

(With 3 Figures in the Text)

A. Introduction

The helminths dealt with in the present paper were recovered from an Indian chukar, *Alectoris graeca chukar*, brought in for examination by Major H. O. Pugh, D.S.O., Glandyfi, Cardiganshire, on 21 April 1938. The bird was one of several which were brought over from India in December 1937, and which were kept in captivity up to the date of examination of the bird under consideration. The death of this bird was found to be due to entero-hepatitis caused by *Histomonas meleagris* (Smith, 1895).

It is thought that an account of the parasites of these birds soon after their introduction into this country, when they are still in captivity, will be of particular interest for comparison with future data, should their introduction prove successful under field conditions.

B. List of helminth species recorded

A nematode infestation of the caeca was found associated with the lesions of *Histomonas meleagris* (Smith, 1895), whilst a heavy infestation of tapeworm species was found in the small intestine.

The species recorded were:

Nematoda: (1) *Heterakis gallinae* (Gmelin, 1790). One adult male; located in the caecum.

(2) *Subulura brumpti* (Lopez-Neyra, 1922), Cram, 1926. Fourteen males and eight females; located in the caeca.

Cestoda: (3) *Raillietina* (*Raillietina*) *graeca* n.sp. Thirteen worms; located in the small intestine.

(4) *Raillietina* (*Skrjabinia*) *indica* n.sp. One gravid worm; located in the small intestine.

(5) *Taenia* (s.lat.) sp. A small immature worm; located in the small intestine.

C. Morphology of helminths recorded

(1) *Heterakis gallinae* (Gmelin, 1790)

The one male specimen collected is 9·5 mm. long and 0·32 mm. broad; its caudal extremity is straight with prominent alae, bearing eleven pairs of papillae. The oesophagus (including the bulb) is 920μ long. The spicules are very unequal, measuring 2 and 0·6 mm. The chitinoid ring of the preanal sucker has a diameter of 57·5μ.

(2) *Subulura brumpti* (Lopez-Neyra, 1922) Cram, 1926

The cephalic extremity is slightly recurved dorsally in both male and female; the cephalic alae are transversely striated, extending a sixth of the length of the worm in the female, and a quarter of its length in the male. Three buccal teeth are discernible at the opening of the oesophagus.

Male. The male is 9–11 mm. long and 0·32 mm. broad; its caudal extremity is curved ventrally, bearing five preanal and five postanal pairs of papillae. The length of the oesophagus (including the bulb) is 1·104 mm. The spicules are winged and equal, measuring 1 mm. in length. The non-chitinous preanal sucker measures 258μ in length, and is situated 304μ in front of the cloaca and 644μ from the caudal extremity of the worm.

Female. The female is 18–19 mm. long and 0·5 mm. broad; the straight conical tail is 1·33 mm. long; the vulva is slightly anterior to the middle of the body. A typical ripe egg measures $78 \times 50\mu$.

(3) *Raillietina* (*Raillietina*) *graeca* n.sp. (Fig. 1)

Description. The specimens of this species measure up to 320 mm. in length, with a maximum breadth of 3 mm. The scolex is 213–234μ in diameter, with a rostellum armed with about 100 hooks of typical Davaineid shape, 13μ in length, and arranged in a double row. The suckers are oval and strongly armed, measuring $88 \times 55\mu$.

The neck measures $1{\cdot}2 \times 0{\cdot}14$ mm. and merges into an immature region of length 46 mm. Typical immature segments measure $0{\cdot}37 \times 1{\cdot}8$ mm., with a length-breadth ratio of approximately 1 : 5. This ratio is maintained throughout the mature region, increasing to approximately 1 : 4 towards the posterior part of the gravid worm. Typical mature and gravid segments measure respectively $0{\cdot}41 \times 2{\cdot}05$ and $0{\cdot}6 \times 2{\cdot}25$ mm.

The strobila is traversed by dorsal and ventral excretory vessels of diameter 6 and 25μ respectively; the ventral vessels are connected at the posterior end of each segment by a transverse connective equal to themselves in diameter. The dorsal vessels are similarly connected by a very fine duct of diameter 4μ, which lies immediately dorsal to the ventral connective.

The strobila is protandrous although the testes remain very much in evidence when the female glands are fully developed. The testes number

41–56, and are arranged in two groups, 8–17 being poral and 28–42 aporal. The earliest formed segments, seen in young strobilae, contain 35–40 testes, the normal number of 41–56 being attained in the later formed segments of the mature strobila. They are round in shape, with a diameter of 47–63 μ. Their distribution extends across the breadth of the proglottis beyond the lateral excretory vessels (Fig. 1). Anteriorly they do not extend beyond the anterior border of the ovary. The cirrus-sac is relatively small and oval, scarcely reaching half-way to the ventral excretory vessel of the poral side. It measures $128 \times 64\,\mu$. The cirrus is unarmed, its protruded portion measuring $160 \times 13\,\mu$. When withdrawn it is slightly coiled within the cirrus-sac, but its eversion causes the coils to be straightened out. The vas deferens is closely coiled, and extends almost to the median line of the proglottis.

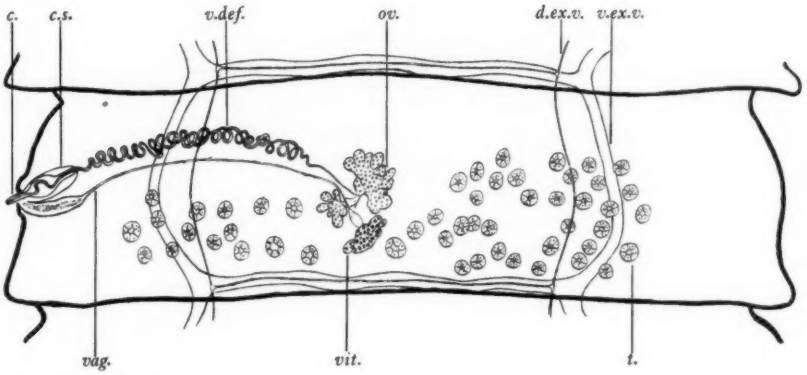

Fig. 1. *Raillietina* (*Raillietina*) *graeca* n.sp. Mature proglottis, 2·05 mm. broad; dorsal view. *c.* cirrus; *c.s.* cirrus-sac; *d.ex.v.* dorsal excretory vessel; *ov.* ovary; *t.* testis; *v.def.* vas deferens; *v.ex.v.* ventral excretory vessel; *vag.* vagina; *vit.* vitellarium.

Both cirrus and vagina open into a common genital pore situated at the middle of the proglottis margin. The genital pores are unilateral, on the left side of the strobila.

The vagina is divided into two portions, the poral portion being a distended sac acting as a functional sperm receptacle (Fig. 1), with a length of 170 μ and a breadth of 32 μ, and the aporal portion being a narrow tube of breadth 6 μ. A receptaculum seminis proper has not been observed.

The ovary is fan shaped, being generally bipartite around the ootype; it consists of 10–14 compact follicles. Its diameter of 260 μ is equivalent to approximately a sixth of the distance between the ventral excretory vessels of the two sides. The ovary is situated slightly poral to the median line of the strobila, its aporal border extending almost to the level of that line. The vitellarium possesses 5–7 blunt lobes, and measures $96 \times 53\,\mu$. It is situated immediately posterior to the ovarian wings.

The uterus extends beyond the lateral water vessels almost to the actual proglottidal margins. It resolves into large numbers of uterine capsules, each with a diameter of 125 μ, and each one containing 8–12 embryos of diameter 15 μ.

Discussion. *Raillietina* (*Raillietina*) *graeca* n.sp. stands fairly close to the two forms *R.* (*R.*) *multicapsulata* (Baczynska, 1914) and *R.* (*R.*) *shantungensis* Winfield & Chang, 1936. It can be readily distinguished from them, however, by the distribution of its testes, which extend beyond the water vessels in *R.* (*R.*) *graeca*, by the extent of its ovary, which occupies a sixth of the median field as contrasted with a half in *R.* (*R.*) *multicapsulata* and a third in *R.* (*R.*) *shantungensis*, and by the fact that each uterine capsule contains 9–12 onchospheres, whilst the other two species have 3 and 1–5 embryos per capsule respectively. Other differences are apparent in the size and extent of the cirrus-sac, the size of the testes, and also in the presence of a distal vaginal distension and a dorsal excretory connective in *R.* (*R.*) *graeca* n.sp.

Out of thirteen specimens of *Raillietina* (*R.*) *graeca* n.sp. recovered from the chukar, twelve were gravid with a uniform length of 265–320 mm. It is noteworthy, however, that one specimen was an immature form of length 75 mm. The existence of this immature worm in a bird that had left India more than five months previously proves that the parasite has been able to find a suitable intermediate host for the completion of its life cycle in this country.

Raillietina (Raillietina) graeca n.sp.

Specific diagnosis. *Raillietina* (*Raillietina*) Stiles & Orlemann, 1926; with generic and subgeneric characters. Length 320 mm., maximum breadth 3 mm. Scolex 213–234 μ broad. Rostellum armed with about 100 hooks, 13 μ long, in a double row. Suckers oval, strongly armed, 88 × 55 μ. Neck 1·2 × 0·14 mm. Typical mature segment 0·41 × 2·05 mm., with length-breadth ratio of 1 : 5. Dorsal and ventral excretory vessels 6 and 25 μ in diameter respectively, both with transverse connectives. Testes 41–56, except in earliest formed segments, where they number 35–40. Testes spherical, 47–63 μ in diameter, extending beyond lateral excretory vessels. Cirrus-sac 128 × 64 μ, scarcely extending half-way to poral water vessels. Everted cirrus 160 × 13 μ. Genital pores unilateral on left side. Vagina with distal distension of 170 × 32 μ acting as a functional sperm receptacle. Ovary of 10–14 follicles, and with a breadth of 260 μ, which occupies a sixth of the breadth of the median field of the segment. Vitellarium with 5–7 blunt lobes, 96 × 53 μ. Uterus extending almost to proglottidal margins; egg-capsules 125 μ in diameter, each with 8–12 eggs, with embryos of diameter 15 μ.

Habitat. Small intestine of *Alectoris graeca chukar*.

Locality and Date. Glandyfi, Cardiganshire, in a bird recently imported from India. 21 April 1938.

(4) *Raillietina* (*Skrjabinia*) *indica* n.sp. (Figs. 2–3)

Description. The length of the strobila is 173 mm., the maximum breadth 3 mm. The scolex is 386 μ in diameter, and bears a retracted rostellum armed with hooks. The rostellar hooks in the one available specimen are absent

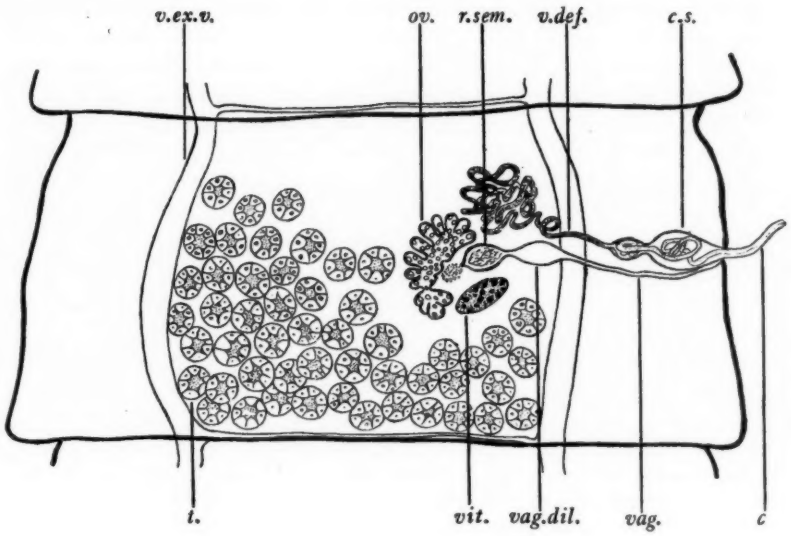

Fig. 2. Mature segment, 1·4 mm. broad; dorsal view

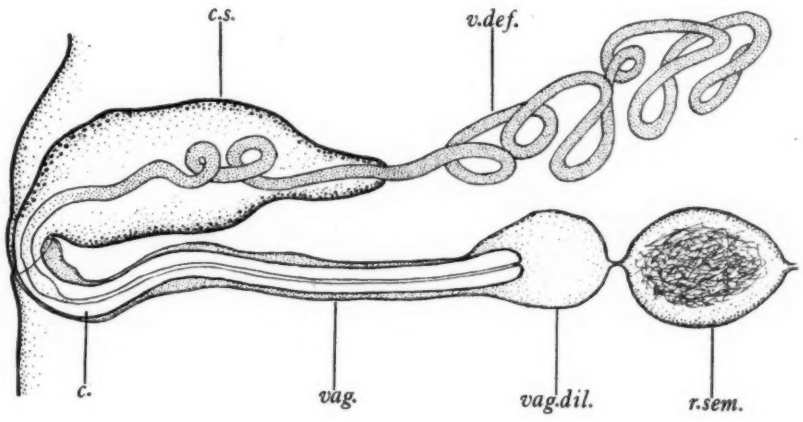

Fig. 3. Diagram showing autoinsemination.

Figs. 2–3. *Raillietina* (*Skrjabinia*) *indica* n.sp.

c. cirrus; *c.s.* cirrus-sac; *ov.* ovary; *r.sem.* receptaculum seminis; *t.* testis; *v.def.* vas deferens; *v.ex.v.* ventral excretory vessel; *vag.* vagina; *vag.dil.* vaginal dilatation; *vit.* vitellarium.

except on a small portion of the rostellar diameter; they measure 13 μ in length, with the typical hammer-like shape. The suckers are round, not very prominent, with a diameter of 117 μ. Acetabular armature is absent, but its absence is probably due to the hooks having been removed prior to mounting.

The scolex is followed by a short neck, measuring 0·9 × 0·14 mm., merging into a long immature region of length 23 mm. The segments of this region are well defined, with a length-breadth ratio of approximately 1 : 3. This ratio increases gradually to 1 : 2 in the mature region, where typical measurements for the proglottids are 0·7 × 1·4 mm. Gravid segments typically measure 0·36 × 0·57 mm., with a length-breadth ratio of about 2 : 3.

The ventral excretory vessels are 106 μ in diameter, and are connected by transverse connectives of 60 μ at the posterior end of each proglottis. Dorsal vessels were not observed.

The strobila is distinctly protandrous, but the testes are still in evidence when the female glands are fully developed. The testes number 52–60 in each proglottis, and are arranged in a wide horseshoe form, extending forwards to the level of the vas deferens aporally, and curving towards the poral water vessels beneath the female glands. They do not extend beyond the excretory vessels on either side. The testes are generally spherical in outline, with a diameter of 75 μ. The typically Davaineid cirrus-sac is pear-shaped, with a greater or less swelling aporally (Fig. 2). It extends almost to the poral excretory canal, and measures 423 × 92 μ; its walls are not very muscular. The cirrus is normally thrown into many coils within the cirrus-sac, a single uncoiled portion connecting the poral and aporal halves of the cirrus-sac. The everted cirrus measures 580 × 32 μ. The vas deferens is coiled, and extends from the cirrus-sac to a distance that is a quarter of the distance between the excretory vessels of both sides. The cirrus-sac opens dorsally to the vagina into a common genital pore. The genital pores are irregularly alternate along the length of the strobila. Several of the segments show a condition of autoinsemination, the cirrus being inserted into the vagina of its own segment, and extending along the whole length of that vagina, almost to the receptaculum seminis (Fig. 3).

The vaginal wall is extremely thin, and after insemination it becomes distended at its aporal extremity to form a large dilatation which acts as a functional receptaculum seminis. This dilatation, whose form is variable (Fig. 3), eventually discharges its sperm into the receptaculum seminis proper. After this discharge is effected the vaginal dilatation remains inflated but empty. When the cirrus is withdrawn the vagina remains as an irregularly collapsed tube. Both the receptaculum seminis and the vaginal dilatation are normally similar in size, measuring about 80 μ in diameter.

The ovary, together with the vitellarium, forms a wide horseshoe whose open end faces the poral margin (Fig. 2). The receptaculum seminis is situated at this opening. The anterior limit of the ovary reaches the level of the posterior coils of the vas deferens. With maturity the ovary acquires a deeply lobed

outline, until eventually it resolves into a collection of follicles. The vitellarium, situated immediately ventral to the receptaculum seminis, is oval in form, measuring $160 \times 85 \mu$.

The uterus extends beyond the lateral excretory vessels almost to the proglottidal margins. It resolves into uterine capsules, each measuring 55μ in diameter, and each containing one egg with an embryo of 32μ diameter.

DISCUSSION. *Raillietina* (*Skrjabinia*) *indica* n.sp. bears some resemblance to *R.* (*S.*) *bolivari* (Lopez-Neyra, 1929) and *R.* (*S.*) *polyuterina* (Fuhrmann, 1909), but can be readily distinguished from them by its larger number of testes, the greater size of its cirrus-sac, and the size of its rostellar hooks.

Raillietina (Skrjabinia) indica n.sp.

SPECIFIC DIAGNOSIS. *Raillietina* (*Skrjabinia*) Fuhrmann, 1920; with generic and subgeneric characters. Length 173 mm., maximum breadth 3 mm. Scolex 386 μ broad. Rostellar hooks 13 μ long. Suckers round, 117 μ in diameter. Neck $0{\cdot}9 \times 0{\cdot}14$ mm. Typical mature proglottis $0{\cdot}7 \times 1{\cdot}4$ mm., with length-breadth ratio of 1 : 2, increasing to 2 : 3 in the gravid region. Ventral excretory vessels 106 μ with connectives of 60 μ. Dorsal vessels not observed. Testes 52–60 in a wide horseshoe, confined to median field. Testes round, 75 μ in diameter. Cirrus-sac $423 \times 92 \mu$, extending almost to poral water vessels. Everted cirrus $580 \times 32 \mu$. Genital pores equatorial, irregularly alternate. Vagina with proximal dilatation adjoining receptaculum seminis. Both dilatation and receptaculum spherical, 80 μ in diameter. Ovary deeply lobed, resolving into a collection of follicles. Vitellarium oval, $160 \times 85 \mu$. Uterus extending almost to proglottidal margins, resolving into capsules of diameter 55 μ, each containing one egg with an embryo of diameter 32 μ.

Habitat. Small intestine of *Alectoris graeca chukar*.

Locality and date. Glandyfi, Cardiganshire, in a bird recently imported from India. 21 April 1938.

(5) *Taenia* (s.lat.) sp.

A small immature Davaineid strobila, 12 mm. in length and 0·78 mm. in breadth, was also recovered from the small intestine. The globular scolex is 690 μ in diameter, and bears four rounded unarmed suckers of diameter 230 μ. The rostellum bears about 200 hooks, 10·6 μ in length, and arranged in a double row.

ACKNOWLEDGEMENTS

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NOTES ON SOME NEW SPECIES OF PARASITIC MITES

By CHARLES D. RADFORD

(With 24 Figures in the Text)

Introduction

Amongst the collections of mites that have been sent to me for identification I have recently found six new species which are described and figured in the present paper.

From Mr G. H. E. Hopkins, Entomologist, Kampala, Uganda, I received for identification a large quantity of the parasitic mites which had been collected in Uganda, chiefly from rodents. One of the tubes contained a number of mites taken from the ears of a waterbuck (*Kobus defassa ugandae* Neumann), and were thought to be a new species of the genus *Raillietia* Trouessart, 1902. Micro-slides of the sexes were forwarded to Dr A. C. Oudemans, Arnhem, Netherlands, who kindly examined them and confirmed my belief, giving a number of references to previous papers on the genus.

Dr C. S. Turnbull found a number of mites in the ears of an ox which he sent to Prof. Leidy, who described them as *Gamasus auris*. This paper (Leidy, 1872) is very brief and the three drawings are of little use for identification of the genus or species.

Apparently this species is the only one that has been described to date, but it is thought that there may be others if the ears of other animals are given careful examination.

Mr H. Britten, Entomologist, Manchester Museum, passed over to me for examination some wrinkled-lipped bats (*Nyctinomus brasiliensis* Geoffrey) from Jamaica which had been forwarded by Mr John Oldham. The mites found on these bats were obviously a new species of the genus *Liponyssus*—the chief characteristic of the male being the large strong spur on each of the fourth pair of legs.

From Mr D. H. S. Davis, Wellcome Bureau of Scientific Research, London, I have received his enormous collection (300 odd tubes) of mites, taken from rodents in Sierra Leone, and in one tube found a *Myobia* nymph. The host was the brown rat (*Rattus norvegicus* Berk.). Although the species is raised upon the single specimen, a nymph, it is considered justified on account of the peculiar arrangement and shapes of the dorsal spines. From the examination of several hundred specimens of this genus, as spirit specimens or micro-slides, it has been found that the nymph is closely similar to the adult female in the arrangement and shape of the dorsal spines. It is hoped that at a later date

the adult sexes will be found, when it will be possible to describe and draw the spines of the female and male.

Mr R. J. Whittick, Department of Zoology, British Museum (Natural History), London, has forwarded fifty-eight slides of *Myobia* from the National Collection for identification. Amongst these specimens three new species have been found, all being from British species of bats.

To the above gentlemen, and to the authorities of the Natural History Museum, I wish to express my thanks for the material given or loaned to me for the production of this paper.

I should be pleased to have the opportunity of examining any species of bat (indigenous or exotic) and specimens of the smaller rodents and insectivores, or mites taken from them.

Genus **Raillietia** Trouessart, 1902

Raillietia hopkinsi sp.n.

The ventral surface of the male (Fig. 1) shows the shape of the ventral shields which appears to be the sternal and genital shield fused into one. The sexual pore is situated at the anterior end of this shield and there are five pairs of spines placed as shown. The mandibles are protruded at the anterior end of the body and are very strongly chitinized.

The second leg is considerably stouter than the others and on the third segment there are two knob-like projections, each bearing a spine. On the posterior edge of the second tarsus will be seen the three oddly shaped spines peculiar to this species (Fig. 3). The hairs on the other legs are all simple with the exception of one at the end of the third tarsus which has a slight bulb-like base. On some mounts this spine may be easily overlooked.

On the tarsus of legs i (Fig. 2) the spines on the dorsal surface are placed as shown. There is a peculiar structure which as far as can be stated is a bifid spine, or two short thick spines which have a common base.

The ventral surface of the male bears only three further pairs of spines with the usual pair on the anal shield, seen at the extreme end of the body. The third, unpaired, seta on this shield is situated at the tip on the dorsal surface.

The tip of the palp is seen (Fig. 5) to possess on the inside edge the usual bifid spine present in the Gamasidae.

The mandibles of the male differ from those of *R. auris* Leidy as described by Hirst (1922) and by Freund (1910). Further, in *R. hopkinsi* the male has no calcarations on the third, fourth and fifth segments of the second legs as has the male of *R. auris* according to Hirst (1922).

The dorsal surface of the female (Fig. 4) shows the position and shape of the dorsal shield, position of the hairs and the pitting or sculpturing. This sculpturing has not been observed in the male. The shield has fifteen pairs of hairs whereas the type species (*R. auris*) has been described as having sixteen pairs by Hirst (1922) and as having twelve pairs by Freund (1910).

The female venter (Fig. 7) shows the position and shape of the sternal and genital shields. Three pairs of hairs are present on the sternal shield and flanking this there is a pair of hairs between coxae iii. The genital shield bears a single pair of hairs and is placed a short distance farther back. Three pairs of hairs are placed between the genital and anal shields. The peritreme is sinuous and extends well forward beyond the first coxae.

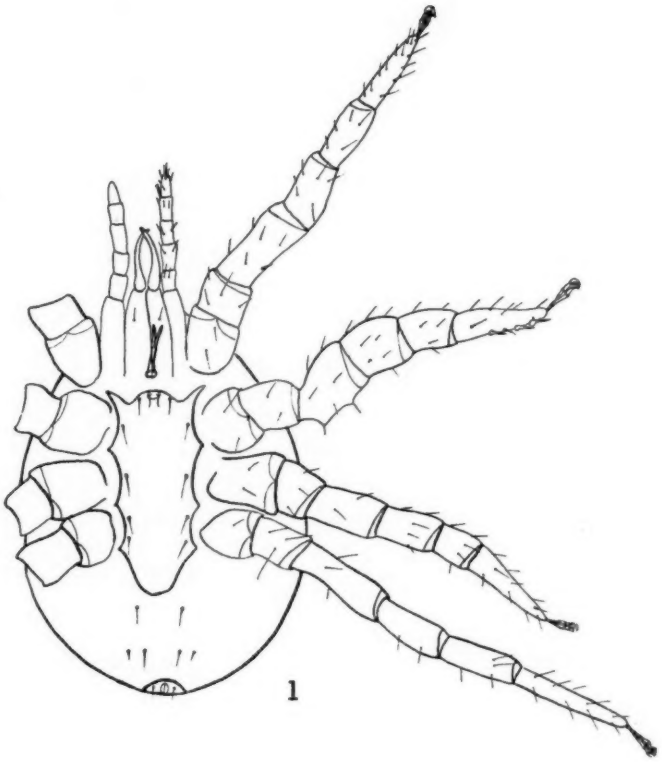


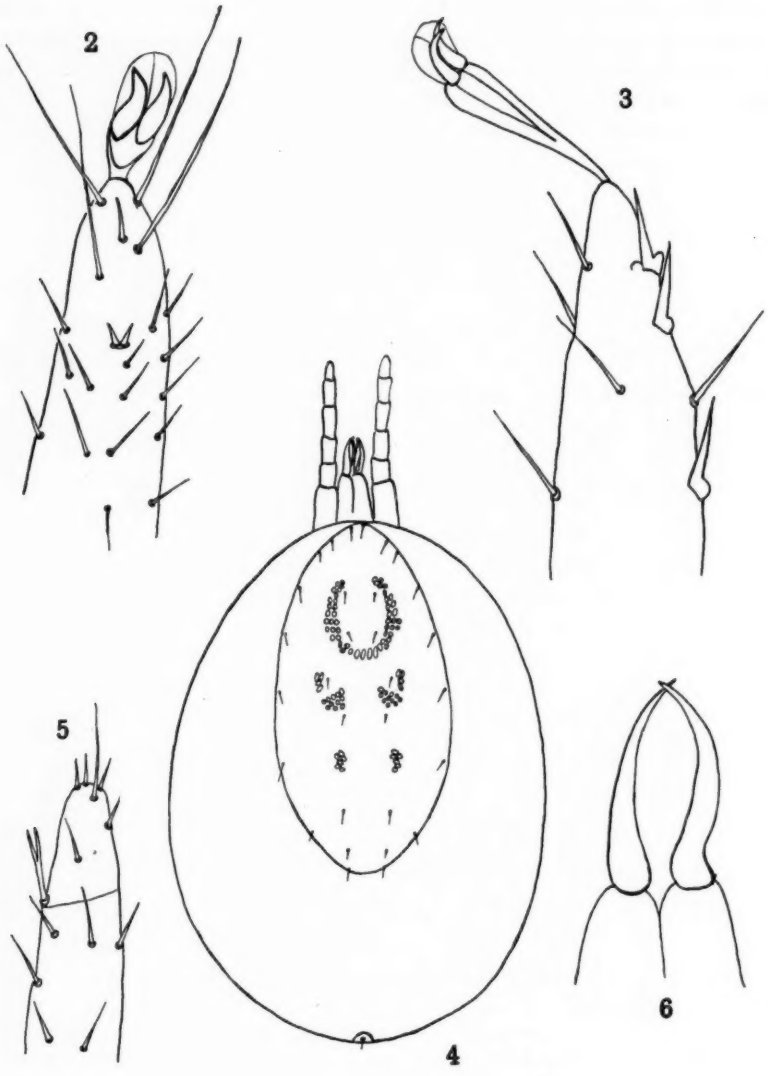
Fig. 1. *Raillietia hopkinsi* sp.n. Venter of male.

The legs of the female are similar to those of the male with the exception of legs ii which are not enlarged or calcarate. The three spines of the male tarsus ii do not appear to be present in the female.

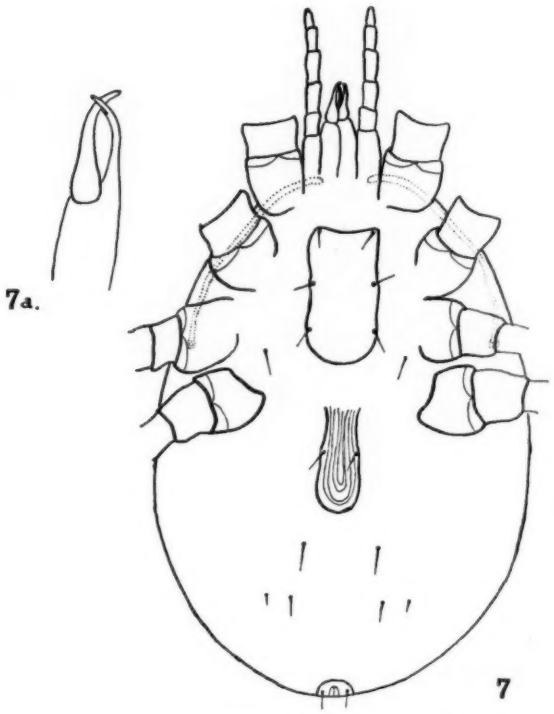
Host: Waterbuck (*Kobus defassa ugandae* Neumann).

Locality: Achwa River, Gulu, Uganda. 21 April 1936.

Measurements: ♀ 0·9 × 0·7 mm. (without capitulum); ♂ 0·67 × 0·5 mm.



Figs. 2-6. *R. hopkinsi* sp.n. 2, dorsal surface of tarsus i; 3, tarsus ii of male; 4, female dorsum; 5, end of palp; 6, male mandibles.



Figs. 7, 7*a*. *R. hopkinsi*. 7, venter of female; 7*a*, mandibles of female.

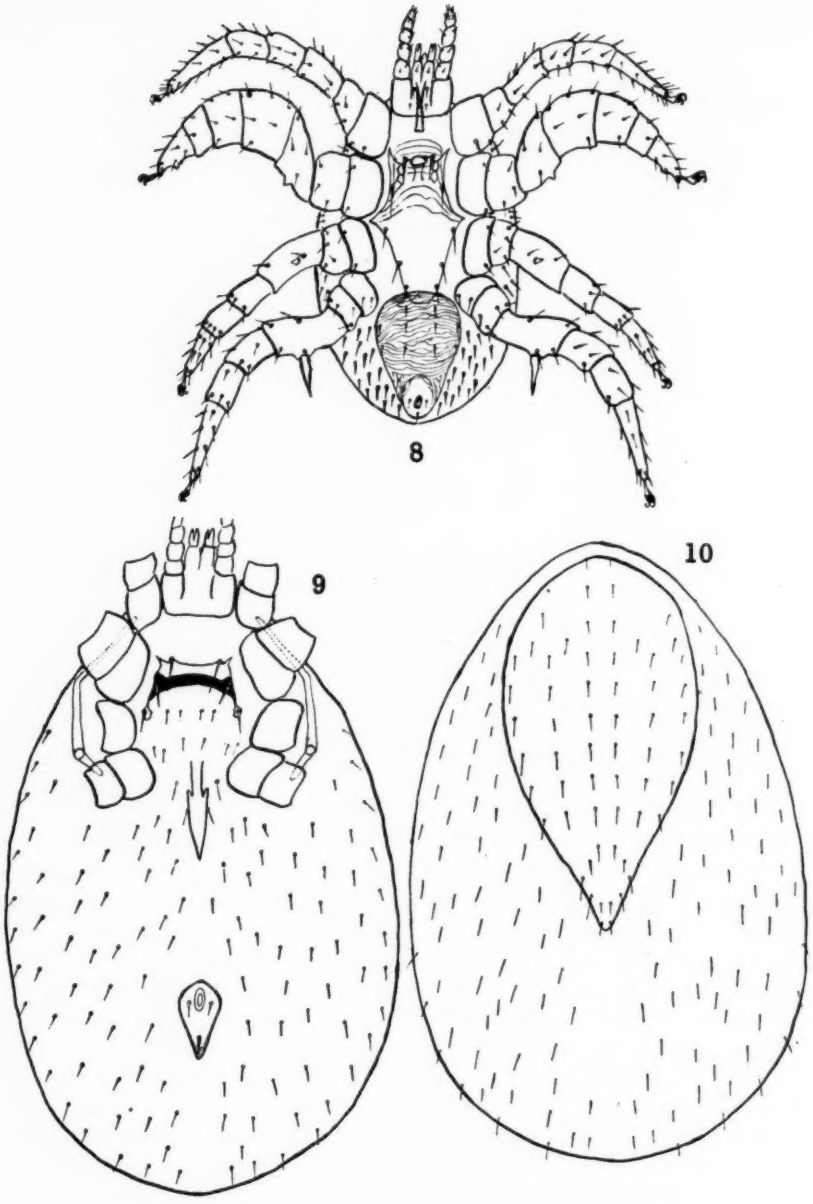
Genus **Liponyssus** Kolenati, 1859

Lyponyssus nyctinomius sp.n.

The venter of the male (Fig. 8) shows the shape and position of the shields and the hairs on the sternal, ventral and anal shields. The exposed portions of the venter possess a number of spines which are shorter than those of the sternal shield. The second legs of the male are seen to be stouter than the others and there is one knob-like calcaration on the third segment, and two on the tarsus. On the ventral surface of legs iii there is a thick spine whilst the third segment of legs iv has a long and stout spur.

The venter of the female (Fig. 9) has the shape common to the engorged females of the genus. The sternal shield has the usual three pairs of hairs, the posterior half of the shield is thickened and the third pair of posterior hairs is situated on small projections of the shield.

The genital shield is shaped as shown in the figure and has a single pair of hairs. The anal shield is bluntly pointed anteriorly and the anal pore is placed at the anterior half of the shield with the paired hairs situated midway between



Figs. 8–10. *Liponyssus nyctinomius* sp.n. 8, venter of male; 9, venter of female; 10, dorsum of female.

the pore and the lateral border of the shield. The third, unpaired, hair is placed well back towards the end of the shield. The venter is furnished with a large number of fine hairs, as also is the uncovered portion of the dorsum.

The dorsal shield (Fig. 10) is elongate pear-shaped and possesses about thirty pairs of fine hairs, situated as shown.

Host: Wrinkled lipped bat (*Nyctinomus brasiliensis* Geoffrey).

Locality: Malvern, Jamaica. 8 May 1936.

Measurements: ♀ 0·83 × 0·52 mm.; ♂ 0·54 × 0·28 mm.

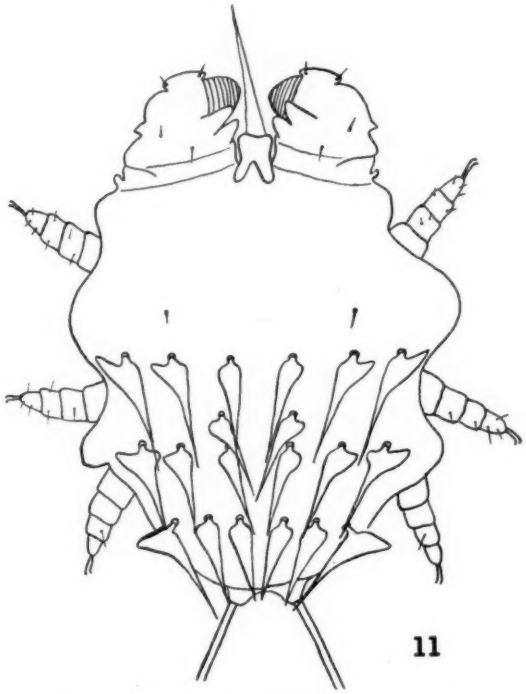


Fig. 11. *Myobia davisi* sp.n. Dorsum of nymph.

Genus **Myobia** v. Heyden

Myobia davisi sp.n.

On the dorsum of the nymph (Fig. 11) there is a remarkable number and arrangement of spines. The first pair of legs consists of the customary grasping organs, and in this case each comprises a broad ventral plate with two finger-like flaps passing across them. The inner surfaces of the processes are grooved to enable the nymph to hold the hair of the host. In addition to these processes the dorsal surface of each of the first legs bears two pairs of small spines as shown.

Midway between legs ii and iii there is situated a single pair of hairs, some distance from the lateral borders. Farther back behind this pair of hairs but slightly in front of the third pair of legs there is a line of six broad spines. Flanking the middle pair of these spines is a somewhat similar pair of spines which are placed about half-way between them and the next row of six spines.

Following this pair of spines there is another line of six broad spines which are not quite the same shape as those of the first series. Behind the level of the fourth pair of legs there is a third series of six broad spines and the posterior end of the body bears the usual terminal bristles.

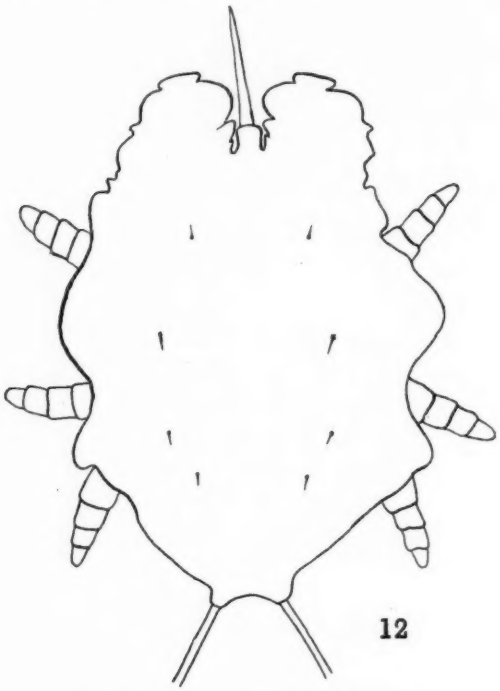


Fig. 12. *Myobia davisi*. Venter of nymph.

As is shown in Fig. 11 the spines in each series are paired; the inner pair of each series being identical or nearly so. The second pair of the series i is somewhat different whilst the outer pair is again different. Series ii are not quite the same in shape as those of series i whilst the spines of series iii, particularly the outer pair, are entirely different.

The venter of the nymph (Fig. 12) shows that there are few spines or hairs. There is a pair between legs ii placed well in towards the centre of the body. Between legs ii and iii there is a larger pair of spines which are wider apart than the first pair. Between legs iii and iv there is a pair of small spines whilst

the fourth pair is closer together and placed on a level with the fourth pair of legs.

Host: Brown rat (*Rattus norvegicus* Berk.)

Locality: Freetown, Sierra Leone. 16 May 1936.

Measurements: Nymph 0·32 × 0·25 mm.

Myobia plecotia sp.n.

On the dorsal surface of the male (Fig. 13) will be seen the usual three outer pairs of spines which are broadly expanded for about half their length, and possess about seven longitudinal striations on their surface. Level with the second pair of legs is the first pair of inner spines, which are not so broad as those of the outer pairs and only extend as far as the third pair of legs. Between legs iii and in line with the first inner pair is a second pair of spines of about equal length and shape. The third pair of inner spines is placed close behind these and is much longer, extending as far back as the end of the third pair of outer spines—not quite touching the lateral margin of the body between legs iv and the terminal end of the body.

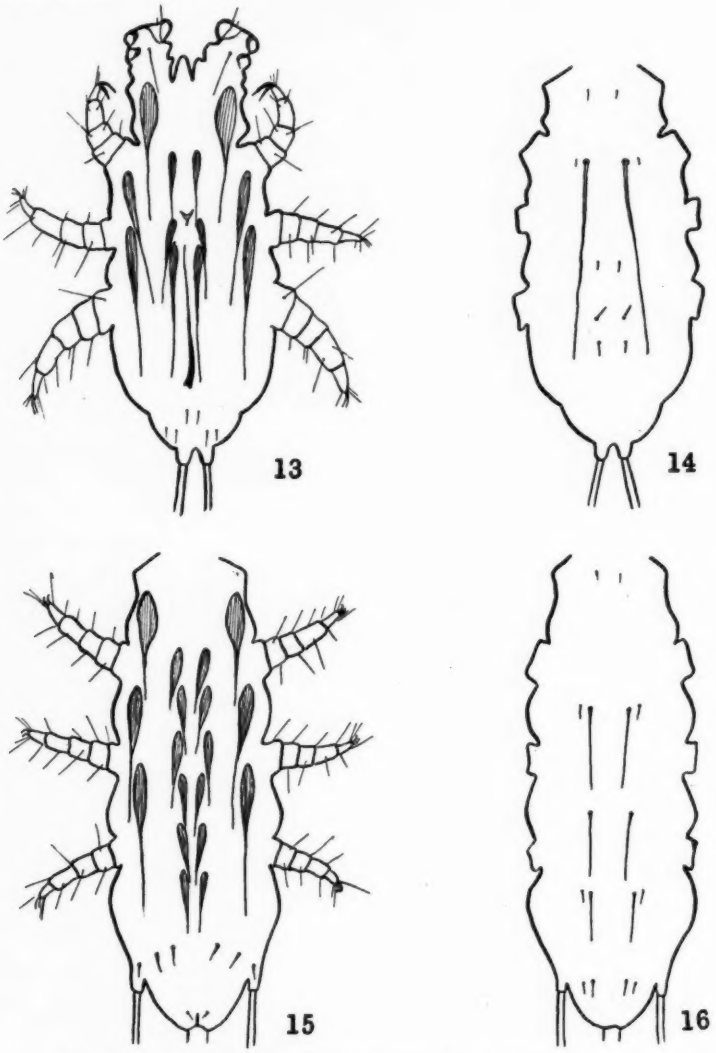
The genital pore of the male is situated between legs iii and slightly in front of the second inner pair of spines. The penis extends from about half-way between legs iv and the terminal end of the body to a point approximately on a level with the third pair of legs. Behind the penis there is a pair of small spines followed by two further pairs of similar spines.

A noticeable feature of this species is the large pair of claws present on the second pair of legs. They are both thicker and longer than is usual in the *Myobia* and noticeably more developed than those of legs iii and iv.

The ventral surface of the male (Fig. 14) shows another noticeable characteristic of this species in the length and shape of the inner pair of spines between legs ii, which reach far backwards towards the terminal end of the body. Between the anterior end of the body and legs ii there appears a small pair of spines near the medial line of the body. Slightly wider apart and between legs ii is situated the long spines of series ii which are flanked by a very small pair. Between legs iii and iv in the middle of the body is a pair of small spines, followed by a second pair between legs iv and a third pair is placed further back behind the last pair of legs.

On the dorsal surface of the female (Fig. 15) the three outer pairs of spines are seen closely to resemble those of the male in shape and position, the third outer pair being shorter than in the male. The first inner pair of spines is placed on a level with legs ii and the second pair slightly behind this pair of legs. In front of legs iii there is the third pair, whilst the fourth pair is placed behind the third pair of legs. The fifth and sixth pairs of spines in this series follow in line and as shown in the figure. There follow three pairs of smaller spines which diverge outwards towards the terminal bristles, and a smaller pair is seen close to the terminal end of the body with another pair at the extreme tip.

The claws of the female are sub-equal, those of legs ii not being enlarged as in the male.



Figs. 13–16. *Myobia plecotia* sp.n. 13, dorsum of male; 14, venter of male; 15, dorsum of female; 16, venter of female.

The ventral surface of the female (Fig. 16) shows that the placing of the spines is not as in the male. At the anterior end of the body there is a single pair of small spines. Midway between legs ii and iii there is a pair of longer

spines flanked by a pair of smaller ones. Between legs iii and iv there is a single pair of fairly long spines, followed by a line of four spines behind legs iv somewhat similar to those of the second series. A line of four small spines is placed on a level with the two long terminal bristles.

Host: Long-eared bat (*Plecotus auritus* L.).

Locality: No data. October 1919.

Measurements: ♀ 0·59 × 0·21 mm.; ♂ 0·44 × 0·21 mm.

This species was also taken on a Pipistrelle bat (*Pipistrellus pipistrellus* Schreb) at Lymore, Hants., 13 May 1919, by the late Stanley Hirst.

Myobia noctulia sp.n.

On the dorsal surface of the male (Fig. 17) the three outer pairs of spines are seen to be somewhat similar to those of the previous species, but there are more striations on them. Between legs ii is the first pair of inner spines with the second pair following in line and on a level with legs iii. Behind legs iii is the third pair of inner spines. Six very small spines are situated towards the end of the body.

One of the outstanding characteristics of this species is the pair of large spines situated at the lateral border of the body behind legs iv, and extending backwards to the extreme tip of the mite.

The sexual pore of the male is situated between legs iii just in front of the second inner pair of spines. The penis, which is almost straight except at the base, arises well back behind the pair of lateral spines and extends forwards to a point level with legs iii.

The claws of this species are sub-equal and are all fairly well developed but not so strong as those of legs ii in *M. plecotia*.

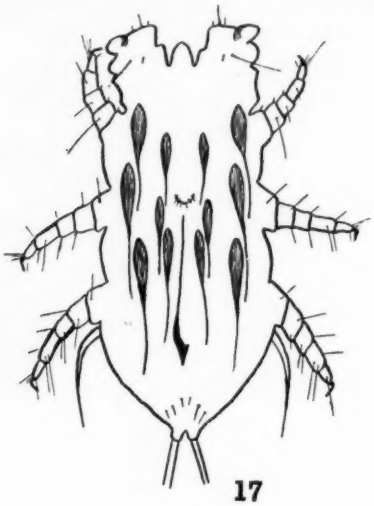
The ventral surface of the male (Fig. 18) shows two pairs of small spines placed before legs ii. Just in front of legs iii there is a line of three pairs of spines, the inner pair of which is long and slender. A similar pair of spines follows, whilst level with legs iv there is a pair of peculiarly shaped spines.

These ventral spines and the lateral spines are two of the characters by which this species can be distinguished easily. The last pair of ventral spines appears to be two-jointed, a feature which is seen again in the three outer pairs of spines in *M. pipistrellia*.

The dorsal surface of the female (Fig. 19) shows the number and arrangement of spines to be closely similar to those of the previous species (*M. plecotia*). The number of striations on the outer pairs of spines is about eleven or twelve, and the last pair of these spines is longer than in the previous species.

The female venter (Fig. 20) shows the arrangement of spines to be different from that of the female of *M. plecotia*. Before legs ii there are the four spines as in the male. Before legs iii there are the three pairs of spines as in the male, and following this line is a pair of spines similar to those of the inner pair. Immediately behind legs iv there is a third pair of these long spines flanked

by a smaller pair. Then follows what appear to be the bases of the pair of spines which are to be found in this position on the venter of the male. As



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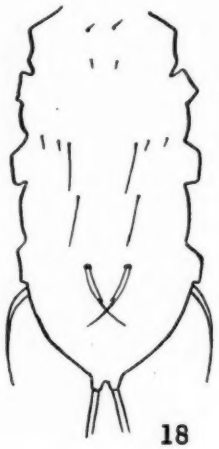

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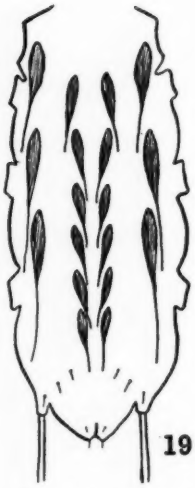

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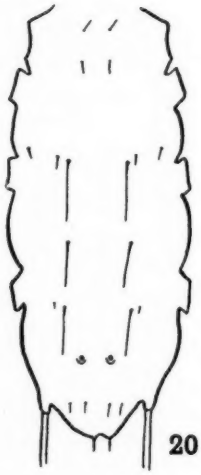

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Figs. 17–20. *Myobia noctulia* sp.n. 17, dorsum of male; 18, venter of male; 19, dorsum of female; 20, venter of female.

there is only one female specimen available for examination I am unable definitely to establish the presence of these spines in the female. From the placing of the marks it seems that the spines should be present but have been

dislodged in preparation. As in the previous species there is a line of four small spines present between the terminal bristles.

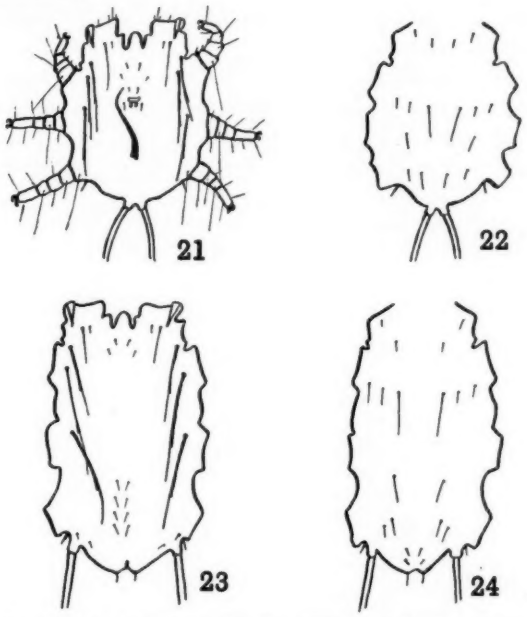
Host: Noctule bat (*Nyctalus noctula* Schreb).

Locality: No data. October 1919.

Measurements: ♀ 0·6 × 0·2 mm.; ♂ 0·5 × 0·2 mm.

Myobia pipistrellia sp.n.

This species cannot be confused with the other known species of the genus owing to its minute size, the stunted shape of the legs, and stout, curved hook-like, claws on the legs.



Figs. 21–24. *Myobia pipistrellia* sp.n. 21, dorsum of male; 22, venter of male; 23, dorsum of female; 24, venter of female.

The dorsal surface of the male (Fig. 21) shows an arrangement of spines not usually found in the bat-infesting species of *Myobia*. Instead of three outer pairs of broad spines there are only three pairs of two-jointed spines, which are slender and apparently unstriated. In the middle of the dorsum and about level with the second pair of legs there are six small spines placed as shown in the figure. Just behind these is the genital pore which is flanked by a pair of small spines.

The penis is stout at the base and gradually tapers to a point and is shaped as shown in Fig. 21.

Behind legs iv there is a small but stout pair of spines at the lateral borders of the body.

On the ventral surface of the male (Fig. 22) is seen the two pairs of small spines before legs ii. Between legs ii and iii there is a line of three pairs of spines of which the inner pair is the longest. Behind this line is a pair of moderately long spines whilst a line of four spines is placed on a level with legs iv.

The female dorsum (Fig. 23) shows the three outer pairs of spines to be two-jointed as in the male and somewhat similarly situated. In the middle of the body there are only four of the six spines present in the male. Four pairs of small spines are placed down the middle of the body between legs iii and the end of the body. Behind the last pair of legs a pair of small stout spines is placed, and on the dorsum there are two pairs of spines placed close to the terminal bristles.

On the female venter (Fig. 24) there are two pairs of spines before legs ii followed by a line of six spines just behind this pair of legs. The centre pair of spines in this line is longer than the rest, and a smaller pair of spines is situated between the last pair of legs. Two pairs of small spines are situated in the median line near the posterior end of the body.

The legs and claws of the female are similar to those of the male.

Host: Pipistrelle bat (*Pipistrellus pipistrellus* Schreb).

Locality: Lymore, Hants. S. Hirst.

Measurements: ♀ 0·32 × 0·18 mm.; ♂ 0·23 × 0·16 mm.

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THE GENERATIVE ORGANS OF THE BLOW-FLY, *CALLIPHORA ERYTHROCEPHALA* L., WITH SPECIAL REFERENCE TO THEIR MUSCULATURE AND MOVEMENTS

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(With 16 Figures in the Text)

In the first part of this paper the male genital organs of *Calliphora erythrocephala* are described, together with the movements of both the internal and external organs. No detailed description of the musculature seems to exist, and Berlese's (1902) account of copulation in *Musca domestica* seems to be the only attempt to describe the movements of the external organs. In the second part the female genital organs are similarly described, and in the third coitus is discussed.

I. The musculature and movements of the male genital organs

(*a*) *The internal organs*

In *Calliphora* the internal organs consist of a pair of pyriform, orange-coloured testes[1] and their ducts, the vasa efferentia, a pair of accessory glands or paragonia, and an unpaired vas deferens, ejaculatory sac and ejaculatory duct. The testis (0·75 × 0·5 mm.), which usually shows an hour-glass constriction at its lower third, is surrounded by an envelope of fat cells, and from it the short vas efferens (0·7 mm.) leads to the distal end of the long vas deferens (1·5 mm.). The accessory gland[2] (1·5 × 0·3 mm.) opens by a very short duct, which accompanies the distal end of the vas efferens into the vas deferens. The vas deferens, which is widest (0·2 mm.) near its commencement, tapers gradually for half its length to become a tube of uniform diameter (0·1 mm.), which opens into the ejaculatory sac, from which the thin ejaculatory duct, 2 mm. in length, passes to the distal end of the phallosome.

Beneath the pigment cells, the whole surface of the *testis* is covered by a single, closely arranged, circularly disposed layer of striated muscle fibres, and this layer is continued along the whole length of the vas efferens.

[1] For the internal organs Lowne's nomenclature is used; for the external that of Patton (1932) and Patton & Cushing (1934) to a large extent.

[2] In *Musca domestica* (Fig. 15, IX) and some species of *Muscina* and *Mydaea* there are no differentiated accessory glands, but the vas deferens is thick and long. In *Fannia* they are globular, in *Sarcophaga* small and in *Scatophaga* very small. In examples of all these genera movements in the lower part of the testis and in the vas deferens have been seen.

The *vas efferens* is lined by cubical cells with small rounded nuclei and has no chitinous intima. There is a distinct thickening of the muscular layer just before the tube enters the wall of the vas deferens, which is probably a sphincter, keeping the orifice of the vas efferens closed except when the passage of spermatozoa is required.

In specimens anaesthetized with chloroform and dissected under salt solution, movements, due to contractions in this musculature, can be observed. In the upper two-thirds of the testis indistinct, peristalsis-like movements occur occasionally, but in the lower third, which is filled by a mass of spermatozoa, movements are seen in most specimens, though they may not commence for some time after the parts have been exposed. Usually three, four, or more waves of contraction follow each other in rapid succession towards the lower end of the testis, driving a shimmering mass of spermatozoa into the upper part of the vas efferens, where it moves backwards and forwards in relation to the muscular movements in the testis. After a short pause the movements are repeated, and this intermittent series of movements may continue for an hour or more. By pricking the testis near the origin of the vas efferens the rate of the contractions may be greatly accelerated, up to 250 per min. The lower third of the testis seems therefore to function as a pump, forcing the developed spermatozoa into the vas efferens. Contractions in the vas efferens have been seldom seen, but occasionally marked peristaltic contractions occur, causing the sudden passage of a mass of spermatozoa into the vas deferens.

The wall of the *accessory gland* consists of an outer capsule, under which is a single layer of thin, transversely placed muscle fibres. The lumen is lined by a layer of large, very tall, columnar cells with hexagonal bases, lying on a basement membrane. Each cell is divided into two distinct and nearly equal portions; an outer portion, which has a distinct, but irregular, inner limit, and contains a large, rounded nucleus, situated near its centre, and darkly staining granular protoplasm, and an inner portion, which is pale and often distended at its inner end (next the lumen) and contains coarser, discrete granules (Fig. 1, III). Near the duct the tall epithelium passes rapidly into cubical epithelium. The secretion lying in the lumen of the gland is white and opaque (except in newly emerged specimens in which no opaque material is seen) and seems in sections to consist mainly of coarse, rounded granules, which stain deeply with haematoxylin. If the gland is pricked or its duct cut across under salt solution, the contents are ejected in the form of a long, white string, which remains as such.

When the gland is greatly distended with secretion the cells are much shorter, and their inner portions are pale and contain very few granules.

The duct of the accessory gland, which is lined by cubical epithelium, is free for a short distance and then passes in company with the terminal portion of the vas efferens of the same side into the proximal end of the vas deferens at a right angle to its long axis. These two ducts travel in an internal projection

of the upper end of the vas deferens nearly to the mid-line, where they open separately (Fig. 1, V).[1]

Not infrequently the accessory glands exhibit peristaltic contractions, with short and irregular periods of quiescence. At intervals portions of the thick, opaque, white contents pour in gushes into the lumen of the vas deferens. Occasionally the contents of the glands are completely expelled, but their

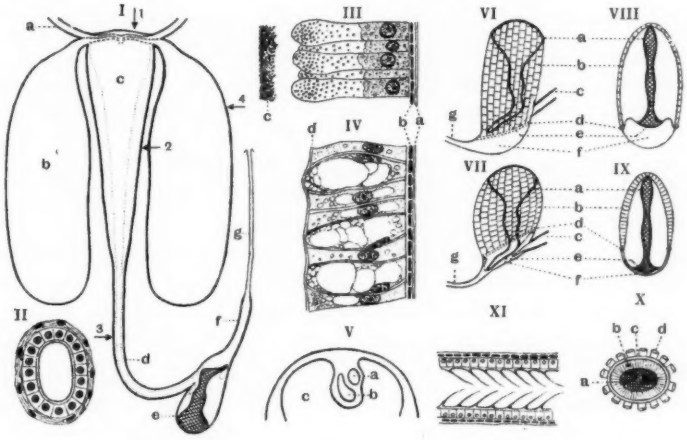

Fig. 1. I. The internal genitalia of the male blow-fly (*C. erythrocephala*), except the testes. *a*. Vas efferens. *b*. Accessory gland. The terminal portions of the vasa efferentia and accessory gland ducts are indicated by broken lines. *c*, *d*. Upper or proximal and lower or distal portions of the vas deferens. The dotted lines indicate the position of the inner membrane. *e*. Ejaculatory sac. *f*, *g*. The muscular and non-muscular (within the phallosome) portions of the ejaculatory duct. II. Transverse section of the vas deferens at arrow 3. III. Section of the wall of the accessory gland at arrow 4. *a*. External capsule. *b*. Muscle layer. *c*. Secretion lying in the lumen of the gland. IV. Section of the wall of the upper part of the vas deferens at arrow 2. *a*. External capsule. *b*. Muscle layer. *d*. Inner membrane. V. Diagrammatic vertical antero-posterior section of the vas deferens at arrow 1 to show the ducts of the vas efferens (*a*) and accessory gland (*b*) running in a fold of the wall. VI. Side view of the ejaculatory sac at rest. *a*. Sclerite (heavy line). *b*. Muscle. *c*. Vas deferens. *d*. Thickened rim of intima (broken lines) to which the muscle is attached. *e*. Base-plate. *f*. Cavity. *g*. Ejaculatory duct. VII. Side view of the ejaculatory sac with the muscles contracted, showing the obliteration of the cavity. VIII. Transverse longitudinal section of the ejaculatory sac at rest. IX. Similar section of the ejaculatory sac with the muscles contracted and the cavity obliterated. X. View of the base-plate from above after cutting through the muscles and stem of the sclerite (black dot). *a*. Oval heavily chitinized part of the base-plate (hatched). *b*. Thin chitin between the base-plate and the thickened ring of intima (*c*). *d*. Muscles (cut short). XI. Diagrammatic longitudinal section of the distal portion of the vas deferens of *Musca domestica*, showing the distally projecting spines.

[1] In *Fannia canicularis* those two ducts enter the globular upper end of the vas deferens in the same manner, but the internal projection is very deep and constricted at its base, so that it has the appearance of a mesentery. In this insect the secretion of the accessory gland is clear, with a few flocculent particles in it, and the globular upper end of the vas deferens appears to be a reservoir only, for it is lined by flattened epithelium. The vas deferens is relatively short.

cavities remain patent and show well-defined margins. Peristaltic contractions in these organs continue for about 2 hours.

The *vas deferens* is thickest at its commencement and decreases gradually in diameter in its proximal half and then, narrowing rather suddenly, is continued to the ejaculatory sac as a tube of uniform diameter.

Throughout its length the wall consists of an outer capsule, internal to which is a single layer of transverse muscle fibres. Internal to the muscle is a single layer of cells lying on a basement membrane. Within the cells is a distinct inner membrane, surrounding the lumen.

In the upper half the cells are columnar with large oval nuclei and darkly staining granular protoplasm, and nearly all contain clear droplets of various sizes. In some cells they are few and small, in others numerous and in others very large. Droplets of considerable size may occur in all parts of the cell, even between the basement membrane and the nucleus. In some cells very large drops or aggregations of droplets are present, which distend the cell and push the nucleus to one side, and sometimes project towards the lumen, bulging the inner membrane. The displacement of the nucleus is best seen in longitudinal sections. These cells appear to be of the nature of goblet cells.[1] The material composing the droplets stains poorly with mucicarmine, but stains deeply with iron haematoxylin. Neither the function nor fate of this secretion has been ascertained. It does not seem, at any rate in the resting state, to pass through the inner membrane, for neither droplets nor uniformly staining material is usually found within it. If the upper part of the vas deferens is cut across and placed in dilute potash solution, the inner membrane with the cells adhering to it is expelled and the capsule left empty (Fig. 1, IV).

In the lower part of the vas deferens the cells, which are cubical with large, rounded nuclei and contain finely granular, darkly staining protoplasm, are closely applied to the inner membrane (Fig. 1, II).

The vas deferens usually shows very active contractions persisting for some hours, often at the rate of one a second, which commonly result in to and fro movements only of the contents. Occasionally, however, after more violent contractions the contents pass into the ejaculatory sac or even through it into the ejaculatory duct.

In the vas deferens of *Musca domestica*, which is relatively very long (Fig. 15, IX), there is a distinct inner membrane, which in the upper half can be dissected out easily and cleanly, but in the lower third is closely applied to the cells. Except in the slightly dilated upper portion, the lumen of the inner membrane remains nearly constant in diameter up to the ejaculatory sac. In its upper third the internal surface of the inner membrane is smooth, but in its lower two-thirds, and especially in its lowest third, numerous distally directed spines project from the internal surface. Each spine is about two-thirds of the diameter of the inner tube in length, has a broad base and tapers to a fine point. The spines, which do

[1] "In some atypical cases (in the epithelium of the conjunction, on the caruncula lacrymalis) the mucinogen accumulates in the whole body of the cell, and the latter, instead of acquiring the form of a goblet, is transformed into a clear oval or spherical vesicle with the compressed flattened nucleus at its base" (Maximow, 1930, p. 40).

not seem to be related to the underlying cells, arise in transverse lines of 6–9 individuals, the lines being about one-third of the length of a spine apart (Fig. 1, XI). Except that in the fly the spines are sparsely placed, the arrangement resembles that of the spines in the proventriculus of the flea. Their function has not been ascertained, but the spines would help to prevent the reflux of masses of spermatozoa in the intervals between muscular contractions.

At the anterior end of the vas deferens the vasa efferentia run in an internal projection to open near the mid-line. The anterior end is lined for a short distance by cubical epithelium, which soon passes into high columnar epithelium, differing in some respects from that found in *C. erythrocephala*, which lines the anterior two-thirds of the tube. The rounded nucleus is usually near the base of the cell surrounded by a small quantity of darkly staining protoplasm, containing small, round, clear droplets. Internal to the nucleus homogeneous, darkly staining globules, some of which attain a considerable size, occur in many of the cells. The inner part of the cell is filled with small clear droplets, which are often coalesced to form large clear areas. Occasionally this part of the cell seems to have been emptied of its secretion. The cell walls are often clearly defined up to the inner membrane, but it is doubtful if they are attached to it for the membrane can be readily dissected away, and in sections may be found separated from the cells, the interval being filled by uniformly staining material. These cells appear to be of the nature of goblet cells. In the resting state the secretion is not found within the lumen of the inner membrane, but during coitus may pass by some means into the lumen, and is perhaps the fluid which distends the lateral sacs of the female.

At the junction of the upper two-thirds with the lower third, the character of the epithelium changes somewhat abruptly and the latter is lined by cubical epithelium, similar to that of *C. erythrocephala*.

Contractions of the testes together with strong and rapid pulsations in the vas deferens, similar to those noted in *C. erythrocephala*, have been seen in *Musca domestica* and in species of *Muscina* and *Mydaea*.

The *ejaculatory sac*, 0·25 mm. length, is a dilatation of the lower part of the vas deferens, on one side of which the intima is modified to form a strong, darkly pigmented, chitinous sclerite,[1] associated with a special musculature. In the wall of the sac the base of the sclerite is expanded into a strong, pigmented, oval base-plate, from the margin of which thinner, less-pigmented chitin spreads outwards to fuse with a ring-like thickening of the intima into which the special musculature is inserted. From the external surface of the base-plate there passes out a strong, rounded stem, which in its distal half is expanded into a large triangular plate, with its long axis parallel to the line of the vas deferens. Very strong muscles pass from the outer margin of the triangular plate to be inserted into the ring-like thickening of the intima. The terminal portion of the vas deferens is very narrow and passes between the muscle bands to enter the anterior end of the sac. When the muscles contract the ventral wall of the sac is drawn up against the base-plate of the sclerite, thus obliterating the sac cavity and driving the contents into the ejaculatory duct. At the same time the terminal part of the vas deferens is compressed, and regurgitation into it prevented. The musculature is so powerful that when it operates the contents of the sac must be expelled with considerable force.

[1] The shapes, sizes and directions of the several parts of the sclerite differ greatly in different species of flies, but in all those examined the structure of the sac is essentially similar.

If the ejaculatory duct is cut across the accessory gland secretion may be expelled from the cut end in the form of a fine thread by the contractions of the vas deferens without contractions in the sac, suggesting that the musculature of the sac is chiefly utilized for discharging the testicular secretion.

The *ejaculatory duct* after a short passage in the body cavity enters the lumen of the phallosome, which is in free communication with the body cavity. Within the phallosome the duct is free until it reaches the distal extremity of that organ, where its wall unites with the external chitin. As far as the base of the phallosome the duct is muscular, but no movements have been noticed in it. Within the phallosome the duct becomes a non-muscular, chitinous tube.

The *ventral nerve cord* sends twigs to the testes and then divides into two branches, which pass ventral to the vas deferens. Each branch sends several small branches to the lower pole of the testis, the vas efferens, the accessory gland, the vas deferens and the ejaculatory sac, and then passes on to supply the external genitalia.

Under the conditions of these observations the passage of the testicular secretion does not synchronize with the passage of the accessory gland secretion into the vas deferens, and some observations indicate, though they are not sufficiently numerous or reliable to afford proof, that the testicular and accessory gland secretions usually pass separately into the ejaculatory sac, the latter passing on without marked contraction of the sac muscle into the phallosome, while the presence of the former excites contraction of the sac musculature, possibly in order to force the spermatozoa into the spermathecae (receptacula seminis) of the female. It seems probable that in coitus the accessory gland secretion is ejaculated first and the testicular secretion later. Whatever the mechanism, there can be no doubt that in the female the testicular secretion passes without much, if any, admixture with the accessory gland secretion into the spermathecae and that the accessory gland secretion passes into the lateral sacs. The fate of the secretion of the vas deferens has not been ascertained.

(*b*) *The terminalia*

The phallosome and the structures most nearly connected with it are situated ventrally in the posterior part of the abdomen. These structures are connected by articulations and muscles with the neighbouring tergites and sternites, which unfortunately have been designated by different numbers by various writers, for there is, as yet, no general agreement as to which segment any given tergite represents. The other chitinous structures have also received different names.

Patton (1932, p. 353) uses the term "terminalia" to indicate "the terminal abdominal segments and their embryonic appendages, which go to make up the genital and anal armatures". He (1932, p. 361) uses the term "phallosome", introduced by Christophers & Cragg (1921), "to indicate the entire intromittent organ in the higher diptera without implying any homologies".

Patton & Cushing (1934) illustrate in a series of papers the terminalia of species of the genera of the Calliphorinae as seen in side view, when mounted in their natural positions in Canada balsam without compression and all drawn to the same scale, in order "(1) to give reliable practical guides for the identification of the species and genera; (2) to reach final conclusions as to the status, affinities and relationships of the various species within each genus, and their true grouping; (3) to fix the limits of each genus in relation to allied genera; and lastly (4) to fix the limits of the sub-family Calliphorinae" (p. 109). These studies do not, however, indicate the functions of the various structures.

The *phallosome* (penis, aedeagus), when at rest, lies in a membranous pocket, the genital pouch. The phallosome is a tube with a wall composed in parts of thin membrane and in parts of thick, pigmented chitin, transmitting within it the terminal two-thirds of the ejaculatory duct. The space within the phallosome, surrounding the ejaculatory duct, communicates proximally with the abdominal cavity, but is closed distally by the fusion of its wall with the wall of the duct. Patton & Cushing (1934, p. 116) divide the phallosome into three parts: (1) A short proximal part (Fig. 2, I) of thick chitin, the bulb of Lowne, carrying a long, bent, posterior process, the spine or epiphallus of Lowne (Fig. 2, *f*), with a smooth lower[1] surface, which during the movements of the organ slides on the roof of the lower portion of the genital pouch. The proximal end of the proximal part has four proximally directed tubercles, the lower pair articulating during the later stages of the rotation leading up to, and during, erection with facets on the lower surface of the apodeme (Fig. 3, *j*). The posterior process of the posterior paramere articulates with and is attached to the lateral depression between the lateral pair of tubercles (Fig. 3, *n*), and a strap-like band of chitin unites the upper pair to the base of the apodeme (Fig. 3, *i*). In the resting position the lower part of the quadrilateral plate is closely applied to the upper half of the proximal part of the phallosome (Fig. 3, *c*); (2) A middle part (Fig. 2, II) mainly composed of membrane, but having "a pair of stout, curved, ventral struts" (Fig. 2, *c*) which are united on both the upper and lower surfaces (Fig. 2, *c''*, *c'''*), and end distally in outwardly directed, pointed barbs. They are the paraphalli of Lowne. On the distal two-thirds of the upper wall is a highly pigmented area, partly covered with short spines, the hypophallus of Lowne or "spined ridge" of Patton & Cushing (Fig. 2, *d*), from which strong lateral processes (*d'*), the cornua of Lowne, project distally, parallel and close to the barbed processes of the paraphalli. In the mid-line a single process, the median spine of Lowne, projects distally, and another projects proximally supporting the membrane (Fig. 2, *d''*, *d'''*); (3) A distal membranous part (Fig. 2, III) strengthened on the upper side by the median distal process of the hypophallus and on each side

[1] The terms upper and lower, in front and behind are used to indicate the relations of the structures when they are in the positions shown in Fig. 8B. This is done in order to avoid the confusion likely to be caused by the use of terms signifying the morphological positions of the structures, presumably attained when the abdomen is completely extended.

by a projection from its cornu. This part terminates in a small, funnel-shaped expansion, at the base of which the ejaculatory duct opens.[1]

There are no muscles within the phallosome, nor any attached to its second or third parts. Presumably, therefore, the parts of the phallosome never change their relative position. According to Lowne (p. 742) "the hooks

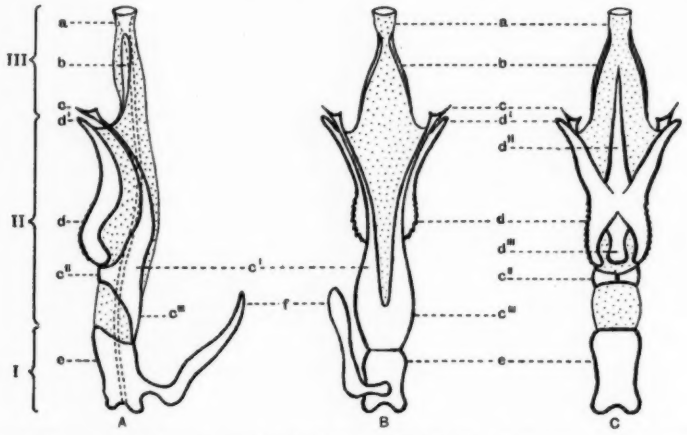

Fig. 2. Phallosome (membranous parts stippled). A. Right side. B. Lower surface. C. Upper surface. I, proximal, II, middle and III, distal parts of Patton & Cushing (1934). *a*. Funnel-shaped free extremity. *b*. Lateral process of the cornu of the hypophallus, supporting the distal part of the phallosome. *c*. Barbed extremity of paraphallus. *c'*. Paraphallus. *c''*, *c'''*. Upper and lower unions. *d*. Hypophallus. *d'*, *d''*, *d'''*. Cornu, median-distal and proximal processes of the hypophallus. *e*. Proximal part of the phallosome or bulb of Lowne. *f*. Spine or epiphallus. In B the spine is represented as turned to one side and shows its smooth, oval, flattened, distal portion, which glides on the roof of the genital pouch. In A the ejaculatory duct is indicated by broken lines.

(or barbs of the paraphallus) probably rest in the pouches (lateral sacs) on either side of the ventral spine of the female during copulation".

The phallosome is attached by a short specialized strap of chitin (Fig. 3, *i*), springing from the upper edge of its proximal part, to the under surface of the apodeme close to its lower end. In the later stages of the rotation of the

[1] The phallosome of *Musca domestica*, which is of much more simple construction, has been carefully described by Patton (1932, p. 361). It consists of a body, apodeme, posterior process and a small paramere on each side, divided into anterior and posterior parts. The distal third of the body, which is membranous, has not been described in detail by Patton. It consists of a curved process, flattened laterally, carrying within it the terminal portion of the ejaculatory duct, which opens on the anterior surface. This process is supported posteriorly by a collar of slightly thicker, but transparent, chitin, attached proximally all round the margin of the heavily chitinized portion of the body, but to the membranous process only along the mid-posterior line. By traction on the ejaculatory sac the membranous process can be withdrawn to a great extent into the body (Fig. 15, IV, A, B, C, D). Whether such a withdrawal plays any part in the act of coitus has not been determined (see p. 473), but it is likely that the collar acts as a piston driving fluid into the lateral sacs of the female.

phallosome leading to erection the lower tubercles of its proximal part rotate in cup-like depressions (Fig. 3, *j*) near the lower end of the apodeme, the strap keeping the parts in contact.

The *apodeme* is a strongly chitinized, slightly curved rod, nearly as long as the phallosome. Its posterior surface has a longitudinal depression and is markedly concave from side to side, whilst its anterior surface possesses a median longitudinal ridge, which divides near the lower end, each branch terminating in a cup-like depression (Fig. 3, *j*). Its upper end is free, and its lower end, besides being attached by the strap to the phallosome, is attached by a short strip of membrane (Fig. 3, *l*) to the quadrilateral plate.

Besides the phallosome, apodeme and quadrilateral plate, there lies in the mid-line, anterior to the latter, the ninth tergo-sternum of Patton & Cushing (1934) or progenital sternum of Lowne. The *progenital plate* is a sheet of chitin divisible into two parts, a body and a shaft (Patton & Cushing). The upper portion or *shaft* (Figs. 3, *a*; 4) is an elongated median plate, with its upper end free and its lower end attached to the *body* (Fig. 3, *b*), a more strongly chitinized and broader plate, showing a well-marked oval, central notch in its lower border (Fig. 4, *d*). On each side of the notch the body articulates with the quadrilateral plate (Fig. 4, *e*), but lateral to the articulation a strongly chitinized prong passes downwards and backwards to form a bent *lateral process* (Figs. 4, *f*; 5, *b*). The lateral process articulates by means of a depression situated a short distance from its extremity with the anterior end of a rod termed the epipleural ridge by Lowne and the proximal segment of the ninth coxite by Patton & Cushing (Figs. 4, *k*; 5, A, *c*). The lateral process also articulates nearer the body, with the extremity of the anterior lateral process of the anal tergum (Figs. 4 near *f*; 5, A, *n*). On this joint the progenital plate is capable of considerable rotation. Across the body there passes a dark crescentic line, along which the membrane of the genital pouch is attached (Figs. 4, *d*; 6 A, *c*).

The median structure here called the *quadrilateral plate* (Figs. 3, *c*; 4, *o*) is attached at its lower end to the lower end of the apodeme by a thin strip of membrane (Figs. 3, *l*; 4). In its upper end is an oval notch, corresponding to the notch in the body of the progenital plate, the two notches bounding an oval membranous area. On each side of the notch the plate articulates with the body of the progenital plate and externally to the articulation is fused with the base of the anterior paramere (Fig. 4). The plate is concave on its lower surface so as to allow it to fit closely to the upper surface of the first part of the phallosome in the resting position. In fact, the lower part of the plate is not visible except when the phallosome is erected (Fig. 3, A′, B′). In the resting position the long axis of the lower part of the plate is almost at a right angle with the long axis of the progenital plate, but when the phallosome is erected the long axes are nearly in line (Fig. 3, A′, B′).

On each side of the quadrilateral plate lies a pair of chitinous structures, the gonapophyses of Lowne or "the two parts of the paramere...completely separated by membrane" of Patton & Cushing (1934, p. 116).

The anterior gonapophysis or anterior part of the paramere, here called the *anterior paramere* (Figs. 3, *d*; 4, *p*), has a wide base fused with the under surface of the quadrilateral plate. It has two processes, (*a*) an anterior in the form of a hollow "long, bent, bluntly pointed plate, flattened laterally with 4–5 short bristles (one apical) along its posterior border", and (*b*) a posterior, which passes downwards and articulates by means of a notch with the anterior process of the posterior paramere (Fig. 3, *m*). From the posterior process a thickened portion of the genital-pouch membrane passes, in the form of a ligament, to the spine of the phallosome (Fig. 3, *h*). In the resting position the anterior paramere lies close against the proximal third of the phallosome, but it does not follow the phallosome in its rotation and is far from it when the phallosome is in the erect position (Fig. 3).

The posterior gonapophysis or posterior part of the paramere, here called the *posterior paramere*, is a long, stout, bent rod, well chitinized except at the apex, where it is membranous. A long stout hair arises from near its base. At its base there are two processes, an anterior articulating with a depression in the posterior process of the anterior paramere and a posterior ending in a sharp point, which articulates with the depression between the lateral tubercles of the proximal part of the phallosome (Fig. 3, *n*). This paramere lies parallel to the spine of the phallosome in the resting position, but during erection remains close to and nearly parallel with the anterior process of the anterior paramere (Fig. 3).

The movements of the phallosome and parameres in relation to the progenital plate.

In the resting position (Fig. 3, A, A′) the progenital plate and apodeme are nearly parallel, and are united by the curved quadrilateral plate, the lower part of which fits closely to the upper side of the proximal part of the phallosome. The phallosome is at right angles to the quadrilateral plate and apodeme, with the anterior paramere lying along its upper side, and the posterior paramere at the side of and parallel to its spine.

If the progenital plate is kept in one position and the free end of the phallosome pushed downwards, so that the phallosome rotates about the joint (Fig. 3, *n*) between its proximal end and the posterior process of the posterior paramere through about 90° (to about the point *o* in Fig. 3), it suddenly springs through a further 50° into the erect position and becomes locked in it, probably by the tension of the ligament (*h*) between the posterior process of the anterior paramere and the spine of the phallosome. During the last part of the rotation the tubercles on the lower side of the proximal end of the phallosome articulate with the cup-like facets (*j*) on the lower surface of the apodeme. At the same time the quadrilateral plate (*c*) becomes straightened, carrying the lower end of the apodeme further from the lower end of the progenital plate and causing the apodeme to lose to some extent its parallelism with the progenital plate. The parameres move closer together, but retain their relationship to the

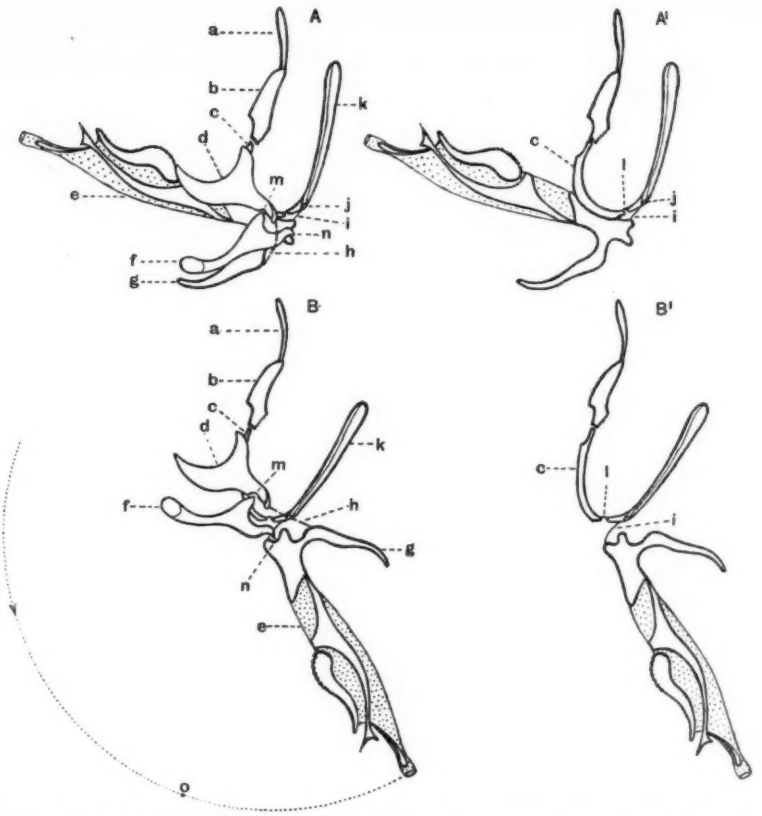

Fig. 3. The movements of the phallosome in relation to the progenital plate. A. Side view of the parts in the resting position. A′. The same with the parameres removed in order to show the position of the underlying quadrilateral plate. B. The position of the parts with the phallosome erected; the progenital plate has been retained in its original position. B′. The same with the parameres removed. *a*. Shaft of the progenital plate. *b*. Body of the progenital plate with its lateral process cut off at the jagged line. *c*. Quadrilateral plate, the lower part of which is shown in A′ to be bent and in close apposition to the upper side of the proximal part of the phallosome. In the erect position (B′) the plate is much less bent. *d*. Anterior paramere; its anterior process projects forward and partly covers the phallosome; its hairs have been omitted; its posterior process projects downwards. *e*. Phallosome. *f*. Posterior paramere lying parallel to the spine of the phallosome in A; its stout bristle has been omitted. *g*. Spine of the phallosome. *h*. Ligament (broken line) connecting the posterior process of the anterior paramere to the spine of the phallosome; slack in A, taut in B. *i*. Strap of chitin connecting the proximal upper edge of the proximal part of the phallosome to the under surface of the apodeme. *j*. Facet on the apodeme with which the tubercle on the lower side of the proximal end of the phallosome articulates in the later stages of the process of erection (the parts are represented as separated in B + B′ so as not to obscure *i*). *k*. Apodeme. *l*. Thin strip of chitin connecting the lower ends of the quadrilateral plate and apodeme. *m*. Joint between the posterior process of the anterior paramere and the anterior process of the posterior paramere. *n*. Joint between the posterior process of the posterior paramere and the proximal part of the phallosome. During erection the phallosome rotates about this joint, the path of its distal end being indicated by the dotted line *o*.

progenital plate and do not follow the phallosome. Clearly they cannot act as claspers during coitus.

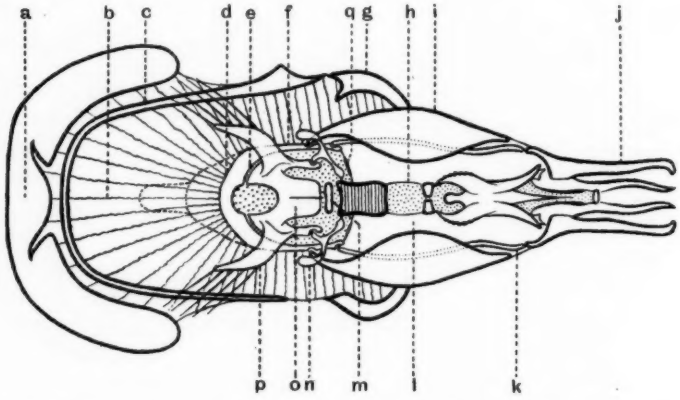

Fig. 4. View of the inner surface of the genital pouch, obtained by drawing the anal tergum backwards and then rotating the phallosome backwards. *a*. Sternum 5, with its lateral lobes. *b*. Genital-pouch membrane passing outwards to be attached to *a*, *c* and *g*, chiefly from the crescentic line (*d*) of the progenital plate. *c*. Rod-like U-shaped sternum 6, articulating with tergum 7 on the left side, but failing to reach it on the right side. *d*. Crescentic line on the body of the progenital plate. The part of the body anterior to the line and the shaft, which lies internal to the membrane, is indicated by broken lines. *e*. Articulation between the quadrilateral plate (*o*) and the body of the progenital plate. *f*. Lateral process of the progenital plate, which articulates with the anterior lateral process of the anal tergum, and, near its free extremity, with the anterior end of the epipleural rod (*k*). *g*. Tergum 7. *h*. Upper surface (the proximal part shaded) of the phallosome (see Fig. 2, *c*). *i*. Anal tergum. *j*. Appendages of the anal tergum. *k*. Distal end of the epipleural rod articulating with the external appendage. Its proximal end articulates with the lateral process of the progenital plate. Its median part, indicated by broken lines, is covered by the infolded margin of the anal tergum. *l*. Ventral wall of the anal tergum, which forms a septum between the genital pouch and the anal fossa. The epipleural rods are thickened portions of this septum. The spine of the phallosome slides on the septum during rotation. *m*. Lower end of the apodeme, joined to the phallosome and to the quadrilateral plate by thickened strips of membrane (see Fig. 3). *n*. Posterior paramere, showing its anterior process articulating with the posterior process of the anterior paramere, and its posterior process with the proximal end of the phallosome. *o*. Quadrilateral plate. The oval membranous area in its proximal border is indicated. *p*. Anterior paramere, with five hairs on its anterior process. Its posterior process is shown articulating with the anterior process of the posterior paramere, but the ligament passing from this process to the spine of the phallosome is not shown. *q*. Irregular moderately chitinized bar connecting the extremities of the lateral processes of the progenital plate, to which the ventral wall (*l*) of the anal tergum is attached.

If the phallosome, when in the erect position, is pushed forwards through about 50° it suddenly springs further forwards and returns along with the other structures into the resting position. This movement is probably due to the recoil of the quadrilateral plate to its normal bent form.

The muscles which bring about these movements are described later (p. 456).

The anal tergum (tenth tergum, Patton & Cushing, 1934, p. 113; progenital (eighth) tergum, Lowne, p. 744, footnote; IX + X terga, Balfour-Browne, 1932).

This is a long, arched plate, triangular when viewed from the side (Fig. 5, A, *e*). Its postero-superior margin is connected in the usual manner by thin intersegmental membrane to the preceding tergum (Fig. 5, B). Its anterior or upper margin is crescentic with the concavity upwards, the anterior horn of the crescent being produced far forwards as a long anterior process, which passes internal to the corresponding angle of the preceding tergum (Fig. 6, A) and ends in a sharp point which articulates with a notch on the side of the lateral process of the progenital plate (Figs. 4, *f*; 5, *n*). About this articulation the progenital plate rotates in a vertical plane. The lateral intersegmental membrane does not arise from the margin of the crescent, but from a more distal crescentic line (Fig. 5, *d*). In the intervening space a strong muscle arises to be inserted into the side of the preceding tergum. This appears to be a flexor of the anal tergum. The lower angle of the anal tergum shows two processes separated by a membranous cleft (Fig. 5, *g*), an inner (*i*) articulating with the bases of the appendages, and an outer (*m*) ending bluntly and in the resting position hiding the epipleural rod. The posterior or lower surface of the anal tergum is incised very deeply from its lower margin to within a short distance of the upper margin (*f*), the incision being bridged by membrane in which the anal opening, with a slightly projecting oval flap on each side of it, is situated (*h*). The distal end of the incision is closed by the bases of the inner appendages (B, *k*). The anterior surface of the anal tergum is composed of membrane connecting the margins of the lateral plates, and forms a septum between the anal fossa and the genital pouch (Fig. 4, *l*). In it occurs a pair of outer rod-like thickenings, the epipleural rods, and a pair of indefinite nearly central thickenings. This septum forms the floor of the lower part of the genital pouch and on it the spine of the phallosome slides.

Appendages articulating with the distal end of the anal tergum.

Two pairs of appendages articulate with the inner process (Fig. 5, *i*) of the distal end of the anal tergum, the inner pair (*k*), termed the anal cerci by Patton & Cushing (1934) and the internal valves by Lowne, and the outer (*j*), termed the distal segments of the ninth coxite by Patton & Cushing, the external valves by Lowne and the cerci or styles by Balfour-Browne (1932).

The inner lie on each side of the mid-line (Fig. 5, B, *k*) and are closely attached together by membrane in their proximal halves, but their distal ends are free and separated. Their proximal attached portions form a swelling, which projects beyond the surface of the posterior aspect of the anal tergum and is covered with numerous hairs. Proximally each articulates by a facet on its outer side with the inner distal process of the anal tergum. Considerable movement in the vertical plane occurs at this joint. Each process is connected on its

inner side to the oval flap at the side of the anus by a thin strand of dark chitin (Fig. 5, *h*), the apodeme of the internal valve of Lowne. Each also articulates for about one-quarter of its length with the inner side of the outer appendage.

The outer appendage (Fig. 5, *j*), of which the distal three-quarters is free, articulates with the inner for about a quarter of its length and also articulates by its proximal end with the outer side of the inner distal process of the anal

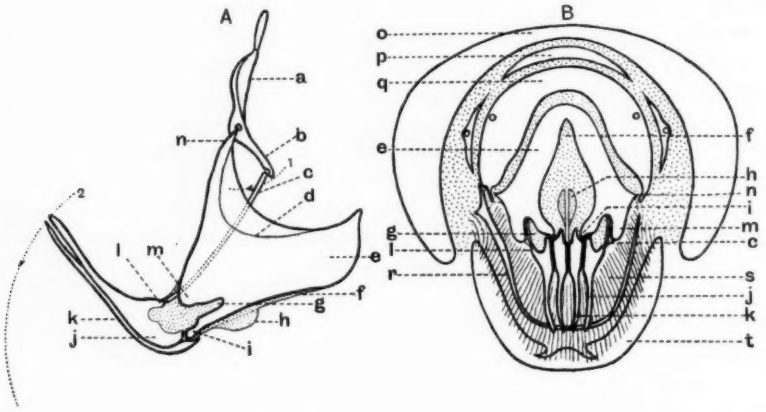

Fig. 5. A. Side view of the anal tergum and progenita plate to show the effect of rotation of the plate on the appendages. B. View of the posterior end of the abdomen of the male from below with the genital atrium partly opened. *a*. Body of the progenital plate. *b*. Lateral process of the progenital plate, showing two articular facets, one near its distal extremity for the epipleural rod (*c*) and the other nearer its base for the anterior process of the anal tergum. The range of movement of the distal end of the lateral process about its articulation with the anal tergum (*n*) is shown by the dotted line 1. When the process moves in the direction of the arrow the rod (*c*) is thrust downwards and extends the appendages on their articulation with the process (*i*) of the anal tergum, so that their tips move to a corresponding extent along the dotted line 2, which indicates the full range of movement, in the direction of the arrow. *c*. The epipleural rod. Its central portion, covered by the infolding of the lateral plate of the anal tergum, is indicated by broken lines (see Fig. 4, *k*). *d*. Semilunar line from which the lateral intersegmental membrane arises. A strong muscle, which flexes the anal tergum on the preceding tergum (*q*), arises from the space between this line and the upper border of the anal tergum and passes to the lateral margin of tergum 7. *e*. Anal tergum. *f*. Membrane covering the deep incision in the lower or posterior surface of the anal tergum. *g*. Membranous cleft separating the inner and outer processes at the lower end of the anal tergum. *h*. Oval membranous flap, lateral to the anus. *i*. Inner lower process of the anal tergum, articulating with both appendages. *j*. Outer appendage. *k*. Inner appendage. *l*. Process of the outer appendage articulating with the lower end of the epipleural rod. *m*. Outer lower process of the anal tergum. *n*. Anterior process of the anal tergum, which articulates with the lateral process of the progenital plate. *o*. Tergum 5 (Patton & Cushing, 1934). *p*. Tergum 6. Its spiracles are situated in thickenings of the intersegmental membrane. *q*. Tergum 7. *r*. Sternum 6; in the form of a strong, U-shaped rod articulating on the left side with tergum 7, but on the right becoming reduced in diameter and failing to reach tergum 7 (see Fig. 4). *s*. Membrane forming the anterior part of the genital pouch (see Figs. 4 and 6). *t*. Sternum 5, showing the central body and lateral lobes. The membranous portions, except the genital-pouch membrane (wavy lines), are stippled.

tergum. Both appendages therefore move synchronously about closely placed joints on this process. On its upper side some distance from its proximal end the outer appendage has a short, strong process (*l*) which articulates with the distal extremity of the epipleural rod (*c*). Traction on the rods helps to flex both pairs of appendages, which cannot move, except to a very small extent, independently of each other, and thrust of the rods is the main factor in extending them. The rods unite the appendages and the lateral processes of the progenital plate, causing the movements of these structures to take place in unison, downwards movement of the lateral processes causing extension and upward movement flexion of the appendages. The tips of the inner processes can be separated to a slight extent and the outer can be abducted from the inner to a slightly greater extent. In the resting position the tips of the appendages embrace the distal end of the phallosome (Fig. 8, A).

The *genital pouch* (Lowne, p. 741) is a sac, chiefly membranous, within which the phallosome lies in the resting position (Figs. 6, A; 8, A). Distal to the base of the phallosome, its posterior wall is the concave undersurface of the anal segment, of which the epipleural rods are differentiated portions (Fig. 4, *l*). Between these, close to the mid-line, lies another pair of less differentiated rods. The upper portion of this wall is attached to the lateral processes of the progenital plate and to a moderately chitinized, irregular bar which unites their extremities (Fig. 4, *q*). From this bar the membrane passes forwards to be attached to the proximal part of the spine and the margin of the proximal part of the phallosome. Anterior to the phallosome the quadrilateral plate and the body of the progenital plate form the median wall of the pouch (Fig. 4). Laterally the wall is membranous and passes from the base of the phallosome to the bases of the parameres, a stronger band on each side forming a ligament connecting the posterior process of the anterior paramere with the spine of the phallosome (Fig. 3, *k*). From a crescentic line on the body of the progenital plate the wall of the pouch is continued as a loose membrane to be attached to the U-shaped sternum 6 (Figs. 4, *b*; 6, *c*). From sternum 6 the membrane is continued as the ordinary intersegmental membrane to the body and lateral lobes of sternum 5.

The *genital atrium* is the opening into the pouch bounded in front by the body and lateral lobes of sternum 5, laterally by the arms of sternum 6 and behind by appendages of the anal tergum (Figs. 6, A; 8).

The intersegmental muscles.

The intersegmental muscles play an important part in extension of the abdomen during the erection of the phallosome and in flexion during its replacement within the genital pouch. Short longitudinal muscles pass between the apposed margins of the terga, an especially strong muscle passing between the anal and the preceding tergum (see Fig. 5, *d*). Longitudinal muscles pass from the anterior margin of each sternum to near the anterior margin of the preceding sternum. Muscles from the extremities of the U-shaped sternum

6 to tergum 7 are strongly developed on the left side, and to a less extent on the right (Fig. 6).

Transverse muscles pass between the lateral margins of the sterna and terga (Fig. 6, B).

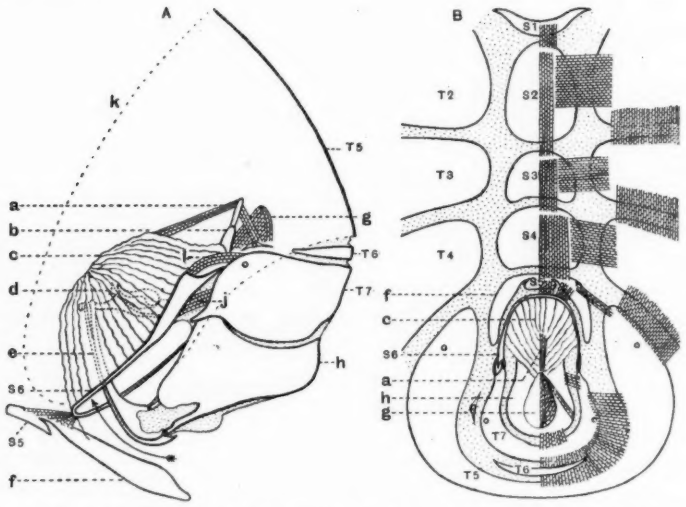

Fig. 6. A. Diagram showing the genital pouch in the resting position, after the removal of tergum 5 on the left side. B. Diagram showing the musculature of the terga and sterna on the right side, as seen from within after the removal of the abdominal contents. *a*. Muscle from the shaft of the progenital plate to the genital pouch membrane. *b*. Body of progenital plate. *c*. Genital pouch membrane, showing its attachment to sternum 6. *d*. Phallosome (dotted) within the genital pouch; its terminal portion is embraced by the appendages. *e*. Appendages of the anal tergum; the portions within the pouch are indicated by dotted lines. *f*. Lateral lobe of sternum 5. *g*. Muscles of the apodeme. *h*. Anal tergum. *i*. Extensor muscle of sternum 6. *j*. Flexor muscle of sternum 6, which produces the final closure of the genital atrium. *k*. The broken line, interrupted at the opening of the genital pouch and passing through *j*, indicates the margin of tergum 5, which has been removed. S. 1–6. Sterna 1 to 6. T. 2–7. Terga 2 to 7. In A the arrow indicates the opening of the genital pouch. Membranous portions, except the genital-pouch membrane (wavy lines), are stippled.

Musculature of the genital apparatus (see Fig. 7).

(*a*) From the posterior part of the internal surface of the anal tergum a flat muscle passes obliquely forwards to be inserted into the middle two-thirds of the epipleural rod.

(*b*) From the anterior angle of the anal tergum a flat muscle passes downwards to be inserted into the middle two-thirds of the epipleural rod on the opposite side to muscle (*a*).

Muscles *a* and *b* presumably act together, causing the rod to move upwards in relation to the anal tergum. This movement pushes the lateral process of the progenital plate upwards, causing the plate to rotate on its articulation

with the anterior process of the anal tergum. At the same time the upward movement of the lower end of the rod flexes the appendages.

(*c*) From the lower posterior angle of the anal tergum a small muscle passes to the integument immediately behind the anal flap and acts as a tensor of the integument round the anus. It probably also helps to extend the appendages by traction on a thin but strong band of pigmented chitin passing from the flap to a process of the inner appendage behind its articulation with the anal tergum (see Fig. 5, B).

(*d*) External to the origin of (*a*) a muscle arises from the side of the anal tergum and is inserted by a tendon into a tubercle on the outer aspect of the inner appendage. In a late stage of the replacement of the phallosome into the genital sac it acts as a flexor, and to some extent as an adductor, of the appendages.

(*e*) From the upper posterior edge of the anal tergum a strong, flat band of muscle passes to the side of the shaft of the progenital plate. Contraction of this muscle rotates the progenital plate backwards on its articulation with the anterior process of the anal tergum, an action which thrusts the epipleural rods downwards and thus extends the appendages. This muscle is an opponent of muscles (*a*) and (*b*).

(*f*) From the upper half of the posterior surface of the apodeme a small muscle passes downwards, to be inserted into the bar connecting the extremities of the lateral processes of the progenital plate (Fig. 4, *q*). This muscle is a tensor of the ventral wall of the anal tergum, on which the spine of the phallosome slides. The ejaculatory duct passes between the muscles of the two sides.

(*g*) From the side of the upper half of the apodeme a strong muscle passes to be inserted along the whole length of the lateral process of the progenital plate. This muscle pulls the apodeme downwards in relation to the progenital plate, and helps to dispose the parts in positions suitable for the erection of the phallosome.

(*h*) Deep to (*f*) a muscle arises from the posterior surface of the lower half of the apodeme and passes to the base of the spine and the sides of the proximal extremity of the phallosome. It extends the phallosome upon the apodeme.

(*i*) A small muscle arises from the side of the apodeme near the junction of its middle and lower thirds and passes to the posterior process of the posterior paramere. It probably fixes the process during movements of the phallosome.

(*j*) A large muscle arises from the side of the upper two-thirds of the apodeme and is inserted into the base and posterior process of the anterior paramere. It probably fixes the paramere during movements of the phallosome and acts as a tensor of the ligament (*m*) (see also Fig. 3, *h*).

(*k*) A strong muscle arises close to the mid-line of the posterior surface of the progenital plate and passes down to be inserted into the upper surface of the lower fourth of the apodeme and the lower third of the posterior surface of the quadrilateral plate. It probably restores these parts to the resting position after erection, its action being opposite to that of muscle (*g*).

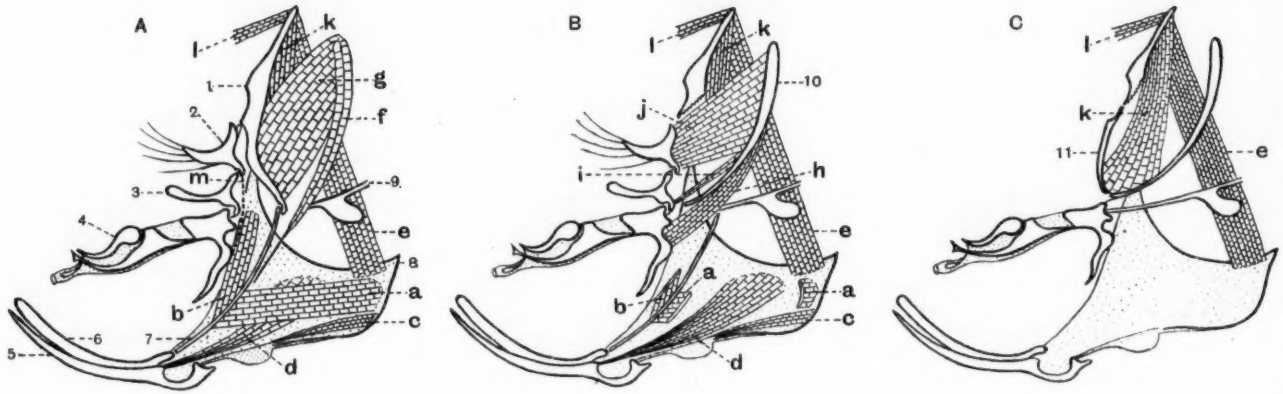

Fig. 7. Diagrams illustrating the musculature of the genital apparatus. A. The musculature as seen after removing the left side of the anal tergum. B. The musculature as seen after removing the lateral process of the progenital plate and muscles *a*, *b*, *f* and *g*. C. Dissection fully exposing muscle *k*. For the origins and insertion of muscles *a–l* see text. *m*. Ligament (broken line) passing from the posterior process of the anterior paramere to the spine of the phallosome. 1. Progenital plate; its lateral process has been removed in B and C. 2. Anterior paramere. 3. Posterior paramere. 4. Phallosome. 5 and 6. Inner and outer appendages of the anal tergum. 7. Epipleural rod. 8. Anal tergum. 9. Ejaculatory sac and duct. 10. Apodeme. 11. Quadrilateral plate (see Fig. 4, *o*).

(*l*) A small muscle passes from the anterior surface of the shaft of the progenital plate to the dorsal surface of the genital pouch (Figs. 6, A, *a*; 8, *d*). It draws the membrane upwards when the phallosome is returning after erection to the resting position. It corresponds to the longitudinal inter-sternal muscles of sterna 2–6.

The erection of the phallosome and its return to the resting position.

By contraction of the longitudinal intersegmental muscles of the sternites and relaxation of those of the tergites the ventral surface of the abdomen is made more concave, and its extremity, particularly by the contraction of the strong lateral muscle passing from the anal to the preceding tergum (Fig. 5, A, *d*), is directed forwards. The atrium is partly opened by the forward movement

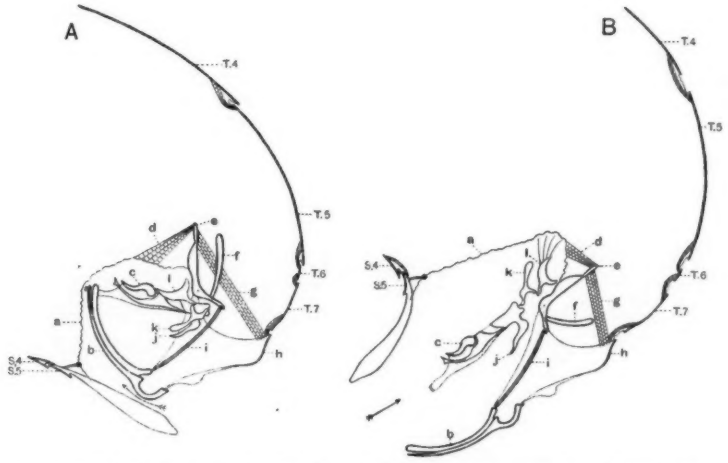

Fig. 8. Diagrams showing the positions of the genital apparatus and the adjacent parts when the phallosome is in the resting position (A), and erected (B). *a*. Genital pouch membrane. *b*. Appendages of the anal tergum. *c*. Phallosome. *d*. Muscle from progenital plate to pouch membrane (see Fig. 7, *l*). *e*. Shaft of progenital plate. *f*. Apodeme. *g*. Muscle from shaft of progenital plate to anal tergum (see Fig. 7, *e*). *h*. Anal tergum. *i*. Epipleural rod. *j*. Spine of phallosome. *k*. Posterior paramere. *l*. Anterior paramere. S. 4–5. Sterna 4 and 5, with the lateral lobe of 5. T. 4–7. Terga 4 to 7. The arrow indicates the opening into the genital pouch.

of the lower end of U-shaped sternum 6 due to the contraction of its extensor muscle (Fig. 6, A, *i*), and then by contraction of the muscle *e* (Fig. 8, *g*) the progenital plate is caused to rotate on the anterior process of the anal tergum (Fig. 5, *n*) until its shaft is pointing backwards (Fig. 8, B). This rotation causes the lateral processes of the progenital plate to thrust the epipleural rods downwards and so extend the appendages of the anal tergum, thus completing the opening of the genital atrium (Fig. 8, B). Next the relation of the apodeme

to the progenital plate is altered by the contraction of the muscle *g* (Fig. 7) and finally the phallosome is rotated and erected by the contraction of muscle *h* and locked in position by the action of muscle *j* on the ligament *m*, and by the change in position of the quadrilateral plate (Fig. 3).

Contraction of muscle *k*, which restores the apodeme and quadrilateral plate to their resting relations with the progenital plate and relaxes ligament *m*, causes the phallosome to rotate forwards into its resting relation with the progenital plate (see Fig. 3 and p. 452). Then the appendages of the anal tergum are flexed by the contraction of muscles *a* and *b*, and their tips caused to embrace the distal portion of the phallosome by the action of muscle *d*. Contraction of the flexor muscle of sternum 6 (Fig. 6, *j*) completes the closure of the genital atrium and relaxation of the longitudinal sternal muscles restores the abdomen to its normal curvature.

II. The musculature and movements of the female genital organs

(*a*) *The internal organs*

The internal female generative organs of *C. erythrocephala* consist of two ovaries, two short ovarian ducts or oviducts leading from them, which unite to form a common oviduct, two long accessory glands, two lateral sacs and three spermathecae.

The *ovaries* in specimens fed from emergence on sugar and water are small, dorso-ventrally flattened, oval structrues (Fig. 9, *s*), and remain throughout life, which may extend to 12 weeks, without showing any appreciable change. In wild specimens, however, the mature ovaries almost fill the posterior half of the abdomen, the alimentary canal, situated dorsally, passing in the V-shaped space between them. The immature ovary is enclosed in a thin capsule, which sends incomplete septa between the egg-tubes, and seems to be continuous with the intima of the ovarian duct. Each egg-tube or ovariole terminates at its upper end in a fine filament and the filaments from the different tubes are collected together to form a kind of suspensory ligament, which passes upwards to the fat body in the region of the heart. Two large tracheae, arising from the spiracles of the 4th and 5th abdominal terga, break up into numerous branches, which enter the dorsal surface of each ovary.

The *ovarian ducts* or *paired oviducts*, which are about one-quarter of the length of the common oviduct, commence as trumpet-shaped extensions of the capsules of the ovaries and unite in the mid-line to form the *common oviduct* (Fig. 9). The common oviduct is about 2 mm. in length when the ovipositor is retracted, but may elongate to nearly double that length when the ovipositor is extended. These three ducts are lined by a thin chitinous intima, lying on flat chitinogenous cells with rounded nuclei. The well-developed muscular coat consists of an inner single layer of stout, closely arranged, circular muscle bands, and an outer layer of less closely arranged, longitudinal muscle bands,

a few of which are continued forwards for some distance on to the capsules of the ovaries.

The common oviduct passes backwards immediately ventral to the rectum as far as the posterior end of the rectal pouch, where it bends sharply downwards to pass into the utero-vaginal tube. The inner surface of the short

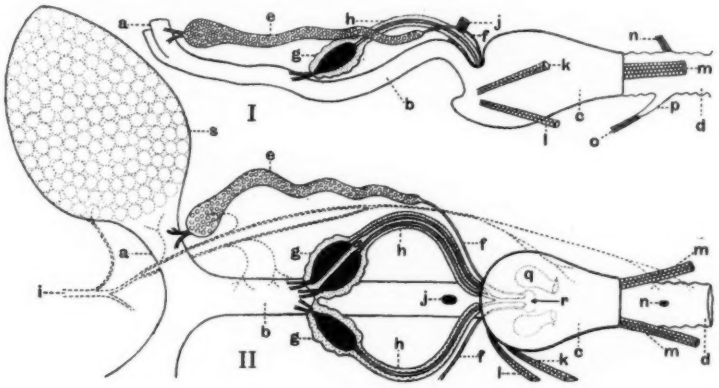

Fig. 9. The female genital organs of *C. erythrocephala*. I. From the left side. II. Dorsal view. *a*. Ovarian duct. *b*. Common oviduct. *c*. Uterus. *d*. Vagina. *e*. Accessory gland, showing rounded projections on the surface due to large cells. It is attached to the ovarian duct by a filament containing tracheae and a few short muscles. *f*. Duct of the accessory gland. *g*. Spermatheca, surrounded by large cells and attached by small muscles to the common oviduct. There are usually two spermathecae on the right side and one on the left. In the mid-line the cells surrounding them meet. *h*. Duct of spermatheca. On the right side the two ducts are enclosed in one muscle sheath. *i*. Termination of the ventral nerve trunk supplying the genital organs. *j*. Origin of muscle passing from the dorsal side of the common oviduct to the ventral side of the third part of the rectum, just distal to the rectal pouch. *k*, *l*. Muscles passing from the distal end of the oviduct to the postero-lateral regions of the second dorsal and ventral sclerites of the ovipositor respectively. *m*. Longitudinal muscle of the uterus passing to the distal end of the third dorsal sclerite. *n*. Origin of small muscle passing from the mid-dorsal line of the vagina to the ventral surface of the third part of the rectum. *o*. Muscle (bilateral) passing from the termination of one of the ventro-lateral diverticula of the vagina to the proximal end of the third ventral sclerite of the ovipositor. *p*. One of the two ventro-lateral diverticula of the vagina. *q*. Cavity of one of the lateral sacs beneath the uterine musculature. *r*. The arrow points to the genital tubercle on the dorsal wall of the uterus. The terminations of the spermathecal and accessory gland ducts passing to it within the uterine wall are dotted. *s*. Abdominal surface of the ovary of a specimen fed throughout life on sugar and water.

downwardly directed portion is thrown into marked folds and the longitudinal muscle layer covering it is modified to form a network (Fig. 10, II, *o*). This part was called the sacculus by Lowne.

Near its posterior end the common oviduct has five extrinsic muscles, a short median muscle passing from its dorsal surface to the ventral surface of the third part of the rectum (Figs. 9, *j*; 11, J), which helps to keep the rectum

in relation to the uterus, and two paired muscles originating in the network, one pair passing dorsally and the other ventrally to the postero-lateral regions of the second tergum and sternum of ovipositor respectively (Figs. 9, *k*, *l*; 12, I, H). These muscles help to keep the uterus and, through the median muscle, the rectum in their correct relationships to the second segment as the ovipositor

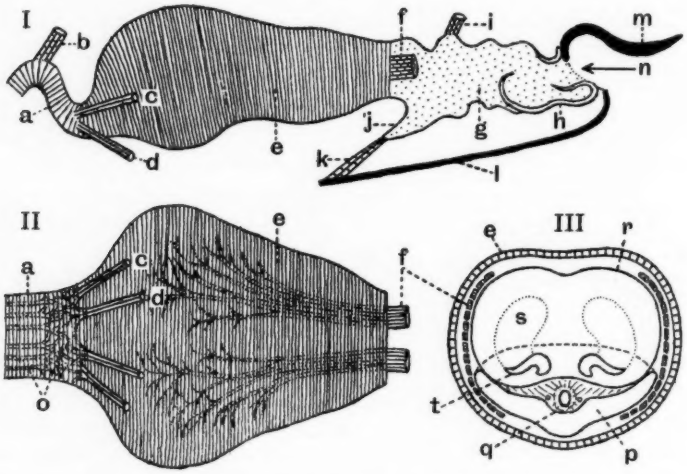

Fig. 10. I. Left side of uterus and vagina to show the musculature. II. Outer surface of the uterus, split down the mid-dorsal line and spread out. III. View of the anterior end of the uterus at the level of the tail of the arrow *r* in Fig. 9. *a*. Common oviduct showing the transverse muscles. *b*. Median dorsal muscle passing to the third part of the rectum. *c* and *d*. Muscles passing to the distal extremities of the second dorsal and ventral sclerites of the ovipositor respectively. *e*. Outer transverse muscle of the uterus. *f*. Inner longitudinal muscle of the uterus passing to the distal end of the third dorsal sclerite. The mode of origin of the longitudinal muscle as branches of the transverse muscle is shown in II. *g*. Chitinous wall of the vagina. *h*. Thickenings in the vaginal wall. *i*. Median muscle passing to the third part of the rectum. *j*. One of the two ventro-lateral diverticula of the vagina. *k*. Muscle passing from the blind extremity of the diverticulum to the anterior extremity of the third ventral sclerite (*l*). *m*. Fourth ventral sclerite of the ovipositor. *n*. Vulva. *o*. Longitudinal muscles of oviduct forming a network from which muscles *c* and *d* originate. *p*. Cavity of uterus. The position of its dorsal wall posterior to the lateral sacs is indicated by the broken line. *q*. Genital tubercle, with the orifice of the common spermathecal duct in the centre and the orifices of the two accessory gland ducts on either side. *r*. Dorsal surface of the thick capsule of the lateral sac. *t*. Sinuous orifice of the lateral sac.

passes from the telescoped to the extended condition. They also aid the longitudinal uterine muscle to draw the uterus towards the vulva in coitus.

The *utero-vaginal tube* is the wide, horizontally directed canal into which the ventral limb of the oviduct (sacculus) passes. The whole of it is lined by a thick chitinous intima, overlying chitinogenous cells, the darkly staining nuclei of which make a very conspicuous layer in sections (Fig. 11, *k*, *m*).

The anterior two-thirds of the utero-vaginal tube are provided with an

outer layer of circular muscle (Figs. 10, *e*; 11, K). This forms a loose sleeve surrounding the chitinous layer. This muscle can be very easily reflected forwards, and is not attached to the chitin anywhere except around the entrance of the spermathecal ducts. This part may be termed the *uterus*

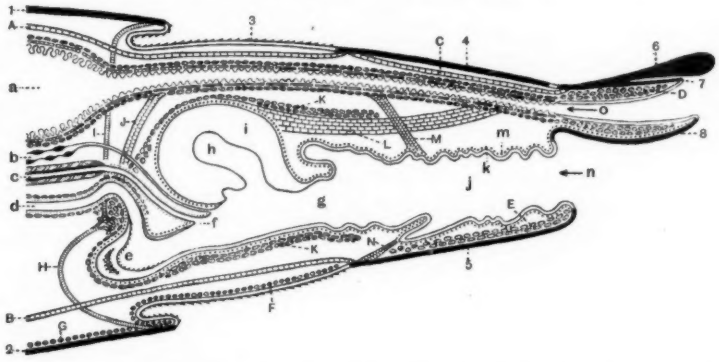

Fig. 11. Diagrammatic longitudinal section of the posterior end of the extended ovipositor of *C. erythrocephala*. 1, 2. Dorsal and ventral sclerites of the second ovipositor segment. 3. Intersegmental membrane. 4, 5. Dorsal and ventral sclerites of the third ovipositor segment. 6. Anal cercus. 7, 8. Dorsal and ventral sclerites of the fourth segment. A, B. Dorsal and ventral longitudinal (retractor) muscles passing from anterior ends of the third to near the anterior ends of the second sclerites (see Fig. 14, *2*). C. Longitudinal muscle passing from the anterior end of the fourth dorsal sclerite to near the anterior end of the third (see Fig. 14, *3*). D. Short muscles of the fourth sclerite. E, F, G. Transverse muscles of the third segment, intersegmental membrane and second segment respectively (see Fig. 14, *17*, *16*). H, I. Ventral and dorsal muscles respectively passing from the distal end of the oviduct to the distal ends of the second ventral and dorsal sclerites. J. Median muscle passing from the dorsal surface of the oviduct to the third part of the rectum. K, K. Transverse musculature of the uterus, continuous with that of the ovipositor. L. Longitudinal muscle of the uterus passing to the distal end of the third dorsal sclerite. M. Median muscle passing from the dorsal surface of the vagina to the ventral surface of the third part of the rectum. N. Muscle passing from a ventro-lateral pouch of the vagina to the anterior end of the third ventral sclerite. *a*. Cavity of the rectal pouch and third part of rectum. The outer circular and inner longitudinal muscle layers and the much folded intima are shown. *b*. Accessory gland and duct. *c*. Spermathecal duct. *d*. Common oviduct. *e*. Median anterior diverticulum of the uterus, with its thickened and spine-covered intima. *f*. Genital tubercle, with the orifices of the common spermathecal duct and one accessory gland duct. *g*. Cavity of the uterus. This ends posteriorly at the level of the letters L, N. *h*. Cavity of lateral sac. *i*. Thick chitinous wall of lateral sac. *j*. Cavity of vagina. *k*. Thick chitinous wall of vagina. *m*. Row of nuclei of cells underlying *k*. *n*. Vulva. *o*. Anus. All the structures shown are bilateral, except J, M, *a*, *d*, *e*, *f*, *g*, *j*, *n*, *o*.

(Fig. 11, *g*). The cavity of the uterus projects anteriorly beyond the entrance of the oviduct as a median diverticulum (Fig. 11, *e*) lined by very thick chitin, showing in its depth a darkly staining reticulum and on its surface short, posteriorly projecting spines. Posterior to the diverticulum the lumen is broad transversely and shallow vertically. On the dorsal surface of its middle third there is a conspicuous rounded tuberosity formed by the centrally fused,

thick capsules of the lateral sacs (Figs. 10, III, *r*; 11, *i*). The orifices of the lateral sacs (Fig. 10, III, *t*), situated on the dorsal wall, are a short distance posterior to the genital tubercle, which is lined by thinner chitin. In the ventral wall, nearly opposite the genital tubercle, a small depression, lined by thinner chitin, is sometimes seen in sections. This depression is more evident in *Musca domestica* (Fig. 15, VI, above lower arrow). It corresponds in position to the mouth of the large incubatory pouch of *Sarcophaga*. Besides the layer of circular muscle, which covers the lateral sacs as well as the body of the uterus, there are inner longitudinal muscles, confined to the sides of the uterus, which commence near its anterior end as branches from the circular layer (Fig. 10, II, *f*). Posterior to the uterus these muscles form on each side a strong band, which passes to the distal extremity of the third tergum of the ovipositor (Figs. 11, L; 14, 7). They draw the uterus towards the vulva and cause eversion of the vagina during coitus.

The wall of the distal third of the utero-vaginal tube is entirely chitinous. This portion may be termed the *vagina* (Fig. 11, *j*). The wall of the vagina is thrown into numerous folds, and there is a particularly deep infolding along the mid-ventral line. Near its distal end two short dorsal and two longer ventro-lateral thickenings are developed in the chitin (Fig. 10, II, *h*), which perhaps strengthen it during the eversion which appears to take place during coitus (Fig. 16). A short distance from the anterior end of the vagina two deep, narrow pouches project forwards and downwards from its ventral surface. A strong muscle passes from the extremity of each pouch to the anterior end of the third ventral sclerite of the ovipositor (Figs. 10, *j*, *k*; 11, N). From the dorsal surface, nearly opposite these pouches, a small median muscle passes to the ventral wall of the third part of the rectum (Fig. 11, M). These muscles probably serve to bring the wall back into its normal position after eversion. There is no circular musculature. At the vulva, situated in the ventral intersegmental membrane just behind the third ventral sclerite, the chitinous wall of the vagina becomes continuous with that of the body wall (Fig. 11, *n*).

The musculature of the utero-vaginal tube is almost identical in *Musca*, *Muscina*, *Stomoxys* and *Fannia*.[1]

The two long *accessory glands* or *parovaria* (Lowne) lie on either side of the oviduct (Fig. 9, *e*). The anterior, blind extremity of each is much dilated and is attached to the ovarian duct by an apical filament, containing a few muscle bands and small tracheae. The body of the gland is greyish-white, and by transmitted light has a faintly spotted appearance due to the opacity of the large cells within. The ducts are thin and, after passing through the muscles

[1] Single eggs in a more or less advanced stage of development, some containing moving larvae, have been found not infrequently in the utero-vaginal tubes of wild egg-laying flies, including *Musca domestica*, *Muscina stabulans*, *Stomoxys calcitrans*, *Fannia canicularis* and *C. erythrocephala*. The egg usually distends the tube from the region of the genital tubercle to the middle of the vagina.

between the descending portion of the oviduct and the uterus, open on the genital tubercle on either side of the common spermathecal duct. The glands and ducts are lined by thin chitin lying on small flattened cells with rounded nuclei. The gland is devoid of a muscular coat, but the duct, until it enters the genital tubercle, possesses a circular muscle layer. The gland cells, which are large and project both internally and externally, possess large round nuclei. They can be dissected off in the form of a sheet from the underlying small cells. The secretion is viscid, slightly granular and highly coagulable, and appears to be the substance which causes the eggs to adhere to one another and to the substratum on which they are laid. In some flies, including species of *Fannia* and *Sarcophaga*, these glands are relatively short and stout.

The three *spermathecae* (*receptacula seminis*, Lowne) are black, oat-shaped chambers with thick chitinous walls. The inner half of the wall is transversely corrugated and highly pigmented, but the outer half is smooth and transparent. External to the chitinous wall is a single layer of large, presumably secretory, cells, except at the posterior end near the origin of the duct, where the large cells are replaced by smaller ones. The spermathecae lie dorsal to the oviduct under the rectal pouch, usually two on the right side and one on the left. They are attached at their anterior ends to the oviduct by small muscles and tracheae. From the posterior end of each chamber a curved duct with a thick, colourless, radially striated chitinous intima, lying on small chitinogenous cells, runs backwards. The single duct on the left and the two on the right, which run side by side, have thick longitudinal or slightly spiral muscle coats. After running for some distance above the oviduct, the spermathecal ducts enter the muscle between the descending part of the oviduct and the uterus, and, losing their muscle coats, unite to form a common duct, which opens by an oval foramen at the posterior end of the genital tubercle (Figs. 9, *g*, *h*, *r*; 11, *e*, *f*).

The spermathecae are for the reception and storage of spermatozoa. In the virgin they contain fluid, presumably secreted by the cells surrounding them, and in the pregnant female closely packed spermatozoa, apparently unmixed with the secretion of the male accessory glands. Possibly the spermatozoa require the cell secretion for their maintenance. In other species examined the spermathecae varied in shape from round to elongated.

The *lateral sacs*, also called the *genital fossae* (Lowne), *conglobate glands* (Patton & Cragg, 1913) and *accessory copulatory vesicles* (Hewitt, 1912), are two elongated cavities surrounded by very thick, transparent, mesially united, chitinous capsules, which form a large tuberosity on the dorsal side of the uterus (Figs. 10, III, *r*; 11, *h*, *i*). The chitin around the cavities stains more deeply than the rest of the capsule. The tuberosity shows a slight longitudinal depression along its dorsal surface, indicating that it represents two lateral structures. The sacs open by means of transversely directed, sinuous, slit-like orifices, placed distal to the genital tubercle on the dorsal wall of the uterus (Fig. 10, III, *t*). The orifices of the sacs seem to be permanently open and are

not connected with the musculature as in the case of *M. domestica*. Presumably the sacs are to some extent constricted and their contents partly evacuated, when necessary, by the contraction of the circular muscle of the uterus and the transverse muscles of the ovipositor. In virgin females the sacs appear to be empty, but in pregnant specimens they contain a quantity of opaque, white, granular material, apparently identical with the secretion of the accessory glands of the male and presumably derived from them (p. 446).

The *genital tubercle* or *spine* (Lowne) is a median invagination of the antero-dorsal wall of the uterus, forming a backwardly directed projection, lined by thin chitin. The common spermathecal duct, which has no muscular coat within the tubercle, opens at its apex at the base of a shallow oval cup, elongated dorso-ventrally, and the accessory gland ducts, which also have no muscular coats within the tubercle, open on either side of the cup (Figs. 10, III, *q*; 11, *f*). In *M. domestica* the genital tubercle is more conspicuous, since its dorsal wall is free from the capsules of the more laterally placed lateral sacs (Fig. 15, VI).

(*b*) *The ovipositor*

The ovipositor or "post-abdomen" consists of several modified abdominal segments, which in the resting state are telescoped within the "pre-abdomen". In *C. erythrocephala* the pre-abdomen is usually regarded as being composed of five segments. There are differences of opinion as to the number of post-abdominal segments, and as to which, if any, have disappeared. The segments of the ovipositor have been regarded as the 6th, 7th, 8th and 9th (Patton & Cragg, 1913), the 7th, 8th, 9th and 10th (Balfour-Browne, 1932), or the 6th, 7th, 9th and 10th, on the assumption that the 8th has entirely disappeared (Awati, 1916; Patton, 1932). Here they are called the 1st, 2nd, 3rd and 4th ovipositor or post-abdominal, without implying any homologies. Except in the case of the last two, the segments are separated by considerable areas of intersegmental membrane, covered externally by minute, backwardly directed spines.

The tergum of the 1st ovipositor segment is saddle shaped and deeply pigmented, and has two spiracles near each lateral margin. Spiracles are not present in the more distal terga. The first sternum is narrow and well pigmented. The 2nd tergum shows between lateral pigmented areas a deep, wide, median, anterior cleft, occupied by smooth, colourless chitin; the sternum is narrow and well pigmented. The 3rd tergum consists of two broad pigmented sclerites, separated from each other in the mid-dorsal line by an unpigmented strip; the sternum is narrow and slightly pigmented proximally, but its distal extremity is well-pigmented and has short lateral cornua. This end forms the ventral edge of the vulva. The 4th tergum is triangular and well-pigmented. Each proximal corner articulates with the inner distal end of a sclerite of the 3rd tergum and a style or anal cercus articulates with each side. The 4th sternum is also triangular, but larger than the tergum. Its proximal corners

are prolonged into long, ventrally curved processes (apodemes, Lowne), which form the dorso-lateral margins of the vulva (Fig. 10, *m*). The anus is situated between the 4th tergum and sternum.

The musculature of the ovipositor consists of (*a*) longitudinal muscles, (*b*) transverse muscles, (*c*) muscles passing from the genital canal to the

Fig. 12. Diagrammatic longitudinal section of the posterior part of the abdomen of *C. erythrocephala*, showing the ovipositor telescoped within it. 1, 1′ and 2, 2′. The dorsal and ventral plates of the last two segments of the abdomen. 3, 3′, 4, 4′, 5, 5′ and 6, 6′. The dorsal and ventral plates of the 1st, 2nd, 3rd and 4th segments of the ovipositor. 7. Anal cercus. The intersegmental membranes exhibit fine spines. R, P, P′, P″ and Q, Q′, Q″. Dorsal and ventral longitudinal retractor muscles of the ovipositor respectively. R, S, T. Ventral transverse muscles of the 3rd, 2nd and 1st segments of the ovipositor and the intersegmental membranes connecting them. U, V. Ventral transverse muscles of the last abdominal segments. In this region the intersegmental membrane is not supplied with transverse muscles. W. Fat body. For explanations of H, I, J, K, L, M, N, and *a*, *b*, *c*, *f*, *g*, *h*, *i*, *j*, *n*, *o* see Fig. 11.

sclerites of the segments and (*d*) small muscles in connexion with the terminal sclerites.

(*a*) The paired dorsal and ventral *longitudinal muscles* are almost in contact along the mid-dorsal and mid-ventral lines. These muscles pass from near the proximal margins of the last abdominal and 1st and 2nd terga and sterna of

the ovipositor to the proximal margins of the 1st, 2nd and 3rd terga and sterna of the ovipositor respectively (Figs. 14, *1*, *2*, *3* and *9*, *10*, *11*; 12, P, P′, P″, Q, Q′, Q″). Muscle 2 has an additional head (2′) arising from the centre of the 2nd tergum. These retractor muscles telescope the segments of the ovipositor within the last abdominal segment. The corresponding dorsal muscles of the abdominal segments are much shorter, but the ventral muscles are similar (see Fig. 6, B and compare Fig. 15, II).

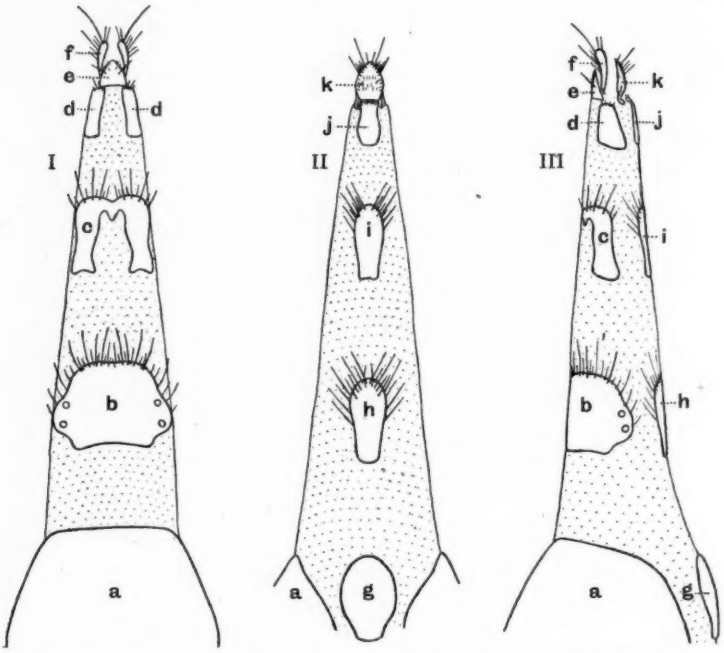

Fig. 13. Dorsal (I), ventral (II) and side (III) views of the extended ovipositor of *C. erythrocephala*. *a*. Last abdominal tergum. *b*, *c*, *d* and *e*. 1st, 2nd, 3rd and 4th terga or dorsal sclerites of the ovipositor. *f*. Anal cercus. *g*. Last abdominal sternum. *h*, *i*, *j* and *k*. 1st, 2nd, 3rd and 4th sterna or ventral sclerites of the ovipositor.

Besides these a muscle (5) passing from the base of the 4th dorsal to the proximal end of the 3rd dorsal sclerite probably flexes the former and another muscle (6) passing from the cornu of the 4th ventral to the proximal end of the 4th dorsal probably flexes the former.

(*b*) The *transverse muscles*, a single layer in thickness, are disposed in three series and confined to the ventral side. Between the sterna they arise from and are attached to the intersegmental membrane, and overlap and partly unite in the mid-ventral line. The most distal series, which consists of thick bands, is confined to the 3rd segment of the ovipositor, where the bands arise from

the inner lateral aspects of the terga and are attached beyond the mid-line to the inner aspect of the sternum. The overlap in the mid-line is very marked (Fig. 14, *17*). The middle series, consisting of much thinner bands, is connected with the 2nd segment and the intersegmental membrane beyond it. It commences a short distance proximal to the 3rd sternum. In the intersegmental region there is marked overlapping and union of the fibres in the mid-line (Fig. 14, III, *16*), but proximally the fibres are attached within the margins of the sternum. In the entire middle series there are about 80 separate muscle bands, of which 44 are confined to the intersegmental membrane.

The proximal series, in connexion with the first segment, is made up of coarser bands. It commences a short distance proximal to the 2nd sternum with curved bands, but the bands gradually become transverse. The series usually extends for a short distance on to the first intersegmental membrane. This series consists of about 75 bands, of which 30 are confined to the 2nd intersegmental membrane (Fig. 14, III, *15*). Extension of this series towards, but not as far as, the last abdominal sternum is not uncommon. In *Lucilia* the proximal series extends to this sternum (compare Fig. 15, II).

All these muscle bands are fan-shaped at their outer extremities and also at their inner, except where the bands of the two sides coalesce (Fig. 14, V).

Increase in the intra-abdominal pressure, mainly by contraction of the transverse ventral abdominal musculature, appears to be the only method for extending the ovipositor, but doubtless its transverse muscles aid in extending its terminal segments. When extended it has, by means of its longitudinal muscles, great mobility in various directions. During oviposition the ovipositor is constantly in motion searching out suitable localities.

(*c*) No muscles pass from the genital tract to the 1st ovipositor segment. Long, thin, paired muscles pass from the distal end of the oviduct to the tergum and sternum of the 2nd segment (Figs. 9, *l*, *k*; 11, I, H; 14, *8*, *13*). These muscles seem to keep the uterus in its proper relation to the ovipositor in all conditions of the ovipositor.

The longitudinal muscles of the uterus pass to the distal margins of the 3rd dorsal sclerite (Figs. 9, *m*; 10, *f*; 11, L; 14, *7*) and draw the uterus towards the vulva and at the same time cause eversion of the terminal portion of the vagina. Muscles pass from the paired ventro-lateral diverticula of the vagina to the proximal margin of the 3rd sternum, and, in conjunction with the muscle passing from the dorsal surface of the vagina to the rectum, help to replace the vaginal wall after eversion (Figs. 10, *k*; 11, N; 14, *12*).

(*d*) Small muscles pass from the 4th tergum to the cerci.

In *Musca domestica* the general arrangement of the female genital organs is similar to that in *C. erythrocephala* (Fig. 15, I), but there are some interesting differences.

The musculature of the common oviduct, probably owing to the relatively great length of the ovipositor and the consequent need for great lengthening and shortening of the duct, is peculiar. The lower part of the capsule of the ovary is covered by a network of fibres, which over the ovarian tubes becomes a single layer of transverse bands. Over the common oviduct from near its commencement to the uterus the muscle bands are arranged in two series of

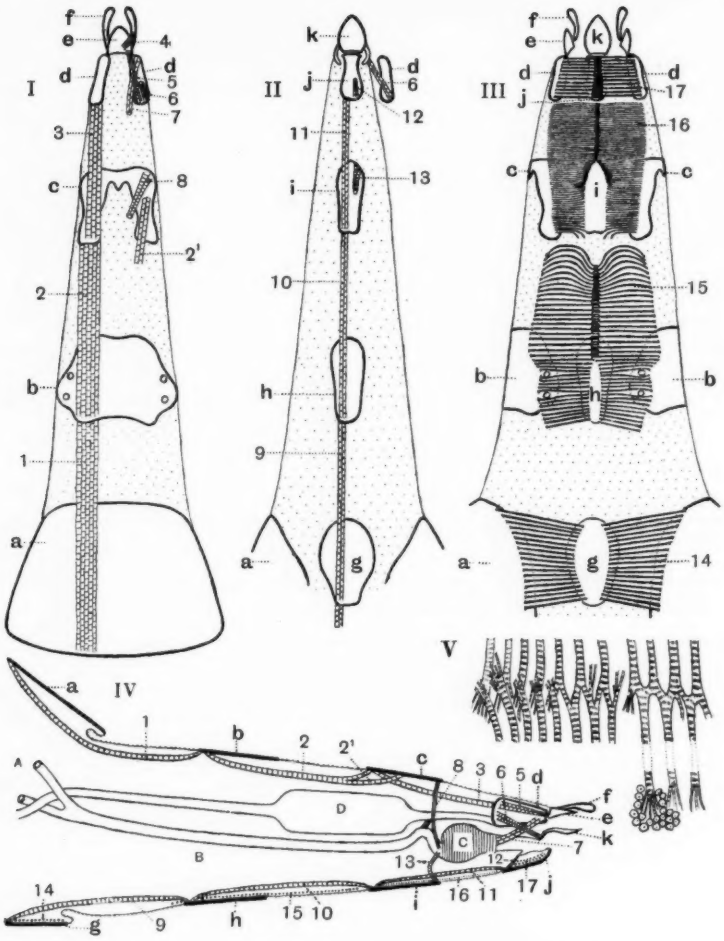

Fig. 14. Diagrams illustrating the musculature of the ovipositor of *C. erythrocephala*. I and II. Inner aspects of the dorsal and ventral surfaces respectively showing the longitudinal muscles. The long retractor muscles are shown only on the left side and other muscles on the right. Each retractor passes from the proximal margin of a distal sclerite to near the proximal margin of a proximal sclerite. III. Inner surface of the ovipositor slit open along the mid-dorsal line and spread out to show the transverse muscles, which are confined to the ventral side. IV. Longitudinal section. V. Modes of fusion and overlapping in the mid-ventral line of muscles of the proximal transverse series. A. Ovarian duct. B. Common oviduct. C. Uterus. D. Rectal pouch. *a–k*. For explanation see Fig. 13. 1, 2, 3. Dorsal retractor muscles of the 1st, 2nd and 3rd segments respectively. 2′. Secondary head of muscle 2, arising from the middle of the 2nd dorsal sclerite. 4. Short muscles of the cerci. 5. Muscle passing from the base of the 4th dorsal to the proximal end of the 3rd dorsal sclerite. 6. Muscle passing from the cornu of the 4th ventral to the proximal end of the 3rd dorsal sclerite. 7. Longitudinal muscle of the uterus passing to the distal end of the 3rd dorsal sclerite. 8. Muscle passing from the distal end of the 2nd dorsal sclerite to the distal end of the oviduct. 9, 10, 11. Ventral retractor muscles of the 1st, 2nd and 3rd segments respectively. 12. Muscle passing to the ventro-lateral diverticulum of the vagina. 13. Muscle passing from the distal end of the 3rd ventral sclerite to the distal end of the oviduct. 14. Transverse muscles passing between the last abdominal tergum and sternum. 15, 16, 17. Proximal, middle and distal series of transverse muscles. In I, II, III the membranous parts are stippled.

semi-circles, with their convexities towards the ovaries, each series almost encircling the tube. The bands of one series pass from near the right mid-lateral line round the tube to near their points of origin, and the bands of the other series pass similarly from near the left mid-lateral line. On the dorsal and ventral surfaces the bands of the two series cross, forming a lattice-like pattern (Fig. 15, VIII). Each band usually arises and terminates in fan-shaped expansions which unite with the convexities of the bands of the opposite series. Occasionally, however, some fibres and more rarely whole bands do not behave in this way, but pass across independently to the other side. This musculature is not attached to the lining membrane of the oviduct, for the latter can be extensively wrinkled without any distortion of the arrangement of the musculature.

The uterine musculature differs from that of *C. erythrocephala* only in the fact that the ventral extrinsic muscle to the second ventral sclerite arises from the wall of the oviduct some distance from the uterus (Fig. 15, I, VI, *b*), and the dorsal extrinsic muscle is related to the lateral sac (Fig. 15, XII, *c*).

The uterus differs mainly in the structure and disposition of the lateral sacs. Distal to the genital tubercle the dorso-lateral wall on each side is expanded to form a pouch, with a thickened chitinous lining, terminating anteriorly in a narrow, tortuous duct, leading to the lateral sac, which is a large thin-walled, chitinous bag, covered externally by flattened cells, lying external to the uterine musculature. The sac is usually empty and its walls extensively folded (Fig. 15, I, XII, *a*), but during coitus it is distended with fluid (Fig. 15, VII, *a*). A median muscle, embedded in the thick chitin, which at the base of the genital tubercle connects the walls of the two dorso-lateral pouches, unites the inner walls of the two ducts, and the dorsal muscle to the 3rd dorsal sclerite of the ovipositor arises partly from its outer wall and partly from the base of the sac external to the uterus (Fig. 15, XII). These muscles probably open the duct when fluid passes in or out. The sac is presumably emptied by contraction of the circular muscles of the ovipositor.[1]

The musculature of the ovipositor is similar to that of *C. erythrocephala*, except that the transverse musculature is much more extensively developed. The transverse muscles extend the whole length of the ovipositor and in most regions, almost completely encircle it. In the 3rd segment the muscles are interrupted only in the narrow spaces between the two rods representing the tergum and the two representing the sternum. In the 3rd intersegmental membrane they are not interrupted. In the 2nd segment they are interrupted only in the space between the two curved sclerites representing the tergum. In the 2nd intersegmental membrane this interruption in the mid-dorsal line is continued, and in the mid-ventral line the bands overlap distally and are narrowly interrupted proximally. In the 1st segment the bands are only interrupted in the space within the fork of the Y-shaped tergum. In the first intersegmental membrane the bands are interrupted somewhat widely in the mid-dorsal and mid-ventral lines. The bands under the intersegmental membranes are much thinner than those in connexion with the sclerites.

[1] The lateral sacs show great variation in shape, size, position and thickness of capsule in different species of house-frequenting flies. In *Protocalliphora* they are of the same type as in *Calliphora*, but relatively smaller and their inner surfaces and orifices are pigmented; in *Pollenia rudis* the capsules are thick, separated anteriorly, but coalesced posteriorly and the sacs are narrow tubes with deeply-pigmented inner surfaces (Fig. 15, X); in *Muscina stabulans* they are narrow, very long, very thin-walled and lie crumpled up under the transverse uterine musculature. They appear to be incapable of more than partial distension (Fig. 15, XI). In *Fannia canicularis* they appear to be absent.

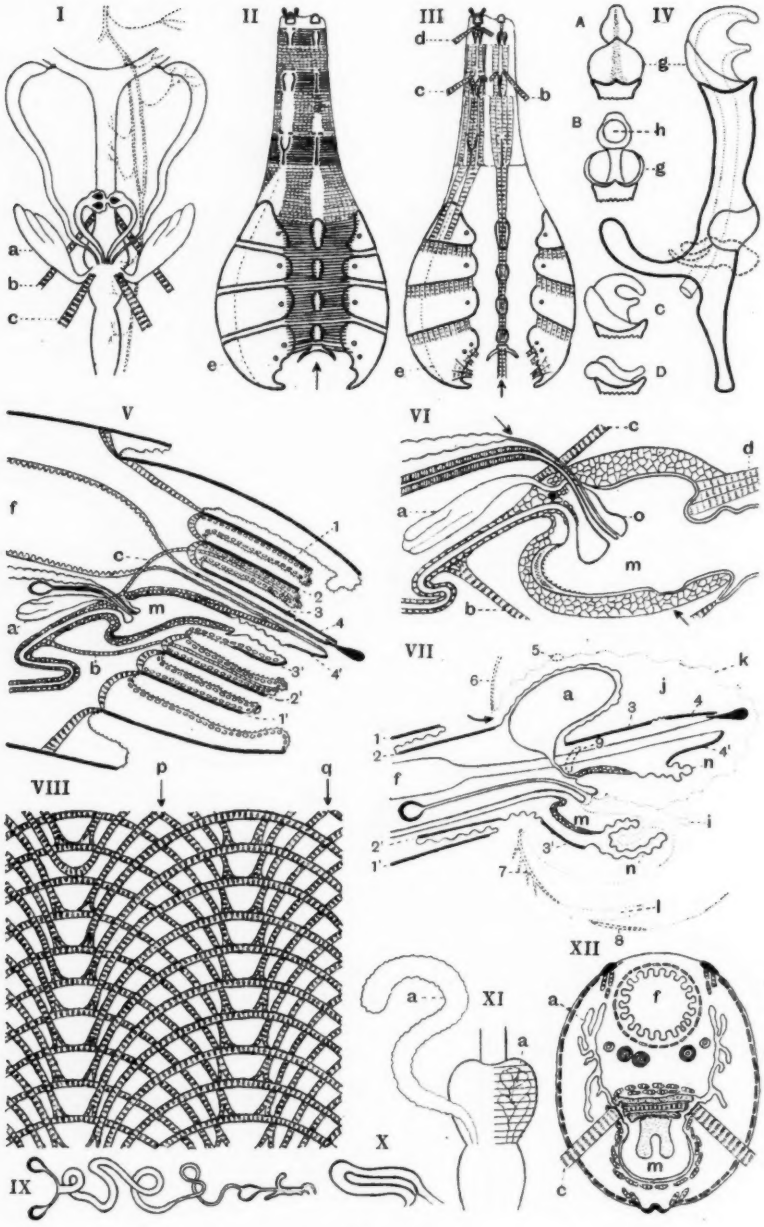
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Fig. 15.

III. *Coitus*

Lowne (1895) and Berlese (1902) were the first to describe in detail the sexual organs of *C. vomitoria* and *M. domestica* respectively. More recently, as an aid to systematic work, the chitinous parts of the male terminalia have been studied by many workers, but only Berlese has investigated the manner

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Legend to fig. 15.

Fig. 15. The figures, except X and XI, illustrate the anatomy of *Musca domestica*. I. Female genital organs, except ovaries (compare Fig. 9, II). II and III. Abdomen and ovipositor slit open longitudinally to show the transverse and longitudinal musculature respectively. In II the thinner intersegmental bands are differentiated. The arrow indicates the mid-ventral line (compare Fig. 14). IV. Side view of phallosome. The ejaculatory duct is indicated by dotted lines and the paramere by broken lines. A, B. Posterior and anterior views of the membranous distal end of the phallosome, showing the opening of the ejaculatory duct (*h*), the collar (*g*), and the strengthened portion in the mid-posterior line (stippled). C, D. Side views of the membranous distal end showing the results of slight and greater traction on the ejaculatory sac. In D the greater part of the membranous portion has been withdrawn into the more strongly chitinized portion. V. Longitudinal section showing the ovipositor withdrawn into the abdomen (see Fig. 12). VI. Longitudinal section of the uterus. The genital tubercle is large, and the lateral sac, which is external to the muscle layer, opens by a narrow duct into a dorso-lateral pouch distal to the genital tubercle. Otherwise the arrangement of the parts resembles that in *C. erythrocephala* (see Fig. 11). VII. Longitudinal section showing the positions of the male and female parts during coitus, based on figures published by Berlese (1902). The male parts are represented by dotted lines. The ovipositor, as far as the distal end of its 2nd sclerite, has been thrust into the genital pouch (*j*) of the male. The lateral sac (*a*) is distended and bulges out the intersegmental membrane between the 2nd and 3rd dorsal sclerites, and the vagina is everted, its ventral wall folding over the posterior process of the phallosome. The ovipositor, proximal to the bulge caused by the lateral sac, is gripped dorsally by the lateral processes of sternum V (1st forceps) of the male, and ventrally between his anal cerci. Distal to the base of the bulge it is gripped by the 2nd forceps (*g*). The distal end of the phallosome is applied to the genital tubercle. The arrow points to the opening of the genital pouch of the male (see Figs. 8; 16). VIII. Middle portion of the common oviduct slit open near the mid-ventral line to show the arrangement of the musculature. Arrows (*p*, *q*) indicate the mid-dorsal and mid-ventral lines respectively. IX. Male genital organs. X. Lateral sac of *Pollenia rudis*. It possesses a thick capsule and a long, tubular cavity with pigmented walls. XI. Dorsal surface of the uterus of *Muscina stabulans* (spermathecal ducts omitted). On the right the lateral sac is shown coiled up under the transverse muscle; on the left the muscle has been removed and the very thin-walled sac extended. XII. Section of the ovipositor of *M. domestica*, at the plane indicated by the arrows in Fig. VI, to show the collapsed lateral sacs outside the uterine musculature and the narrow ducts leading to them from the uterine cavity. A median muscle embedded in the chitin at the base of the genital tubercle passes between the inner walls of the two ducts, and the muscles *c* are attached to their outer walls. Ventral to the rectum the three thick-walled spermathecal and two thinner-walled accessory gland ducts are shown. *a*. Lateral sac. *b*. Muscle from oviduct to 2nd ventral ovipositor sclerite. *c*. Muscle from oviduct to 2nd dorsal sclerite. *d*. Longitudinal muscle of uterus. *e*. Mid-dorsal line. *f*. Rectum. *g*. Collar of phallosome. *h*. Opening of ejaculatory duct. *i*. Phallosome in VII. *j*. Cavity of genital pouch. *k*. Membrane of genital pouch. *l*. Rectum of male in VII. *m*. Cavity of uterus. *n*. Everted vaginal wall. *o*. Genital tubercle. 1, 2, 3, 4 and 1′, 2′, 3′, 4′. 1st, 2nd, 3rd and 4th dorsal and ventral ovipositor sclerites. 5. Sternum 6. 6. 1st forceps of sternum 5. 7. Anal cerci. 8. Anal tergum. 9. 2nd forceps.

of copulation. He described both the preliminary courtship and the act in *M. domestica*, and was able to kill and dissect or section united pairs. He noted that the male stood on the female, but was relatively passive throughout. His dissections showed that when the resting ovipositor was telescoped within the abdomen the rectal pouch, spermathecae and uterus lay within the abdominal cavity, but that when the ovipositor was extruded and erected it increased greatly in volume and that the organs mentioned were drawn into it. He observed that in sexual intercourse the ovipositor was only partly extruded, and that the terminal portion, as far as the second segment, was introduced into and completely filled the genital pouch of the male. After insertion it was seized dorsally by the first forceps (processes of sternum 5) and ventrally by the second forceps of the male.[1] He observed that the lateral sacs of the female, which he was the first to describe, became distended with fluid (which he thought might be derived from the oviduct) and caused an extensive bulge of the intersegmental membrane between the second and third dorsal sclerites. He concluded that the ovipositor was held securely in place by the two pairs of forceps, the distension of the lateral sacs, the denticulation of the intersegmental membrane and to a slight extent by the anal cerci of the male, and thought that it was the difficulty in separating rapidly which enabled him to secure united pairs for dissection (Fig. 15, VII). He noted that the vagina was partly everted and was of opinion that the orifice of the oviduct was brought into apposition with the extremity of the phallosome by muscular action within the ovipositor, whereas it is the orifice of the common spermathecal duct, at the apex of the genital tubercle, a structure which he apparently failed to notice, which is guided to the extremity of the phallosome.

As shown previously the walls of the genital sacs, even in egg-laying females, are usually crumpled and in apposition. When the sacs are distended separation of the flies could only occur with difficulty and retraction of the ovipositor with the abdomen would also be difficult, so that it is probable that the fluid only remains in the sacs for a short time. The function of the fluid, apart from distending the sacs, is unknown.

In *Calliphora* copulation has seldom been observed and has never been described. Lowne (1895) states that "it takes place in the sunlight in the early morning. The male is above the female. These insects never copulate on the wing. They are very shy when united and immediately separate when captured." He thinks that the "hooks" of the paraphalli "rest in the pouches (lateral sacs) on either side of the genital spine of the female during copulation".

[1] In anaesthetized males the posterior end of sternum 5 is from time to time rotated downwards so that it projects almost at right angles to the abdominal wall, and the tips of its lateral processes, the first or primary forceps, make grasping motions, approximating and receding from each other. At the same time the atrium of the genital pouch is opened occasionally, the anal cerci make slight grasping movements and the second forceps (coxites of the ninth segment, Patton), which "are obviously very efficient grasping organs" (Patton, 1932), make very marked grasping movements.

The writer has been unable to secure united pairs for examination, but has observed the opening and closing of the genital atrium and the positions assumed by the different parts of the genital apparatus of the male when at rest and when the phallosome is erected, and has noted that the contents of the accessory glands are passed into the ejaculatory sac before the testicular secretion. In the female he has observed the protrusion of the ovipositor and the eversion of the vagina (Fig. 16, *x*) in partially anaesthetized specimens, and has found that after coitus, the spermathecae are filled with spermatozoa and the lateral sacs with fluid resembling the secretion of the male accessory glands.

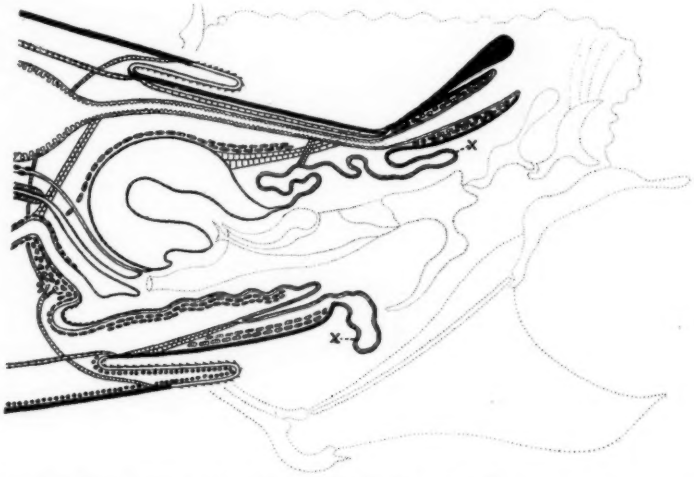

Fig. 16. Diagram illustrating the probable position of the parts during the first stage of coitus in *C. erythrocephala*. The female parts are indicated by solid, the male by dotted, lines. For explanation of the female structures shown see Fig. 11 and male structures Fig. 8, B.

Fig. 16, based on these observations, indicates the probable position of the parts in the first stage of coitus, when the barbed extremities of the paraphalli seem to be inserted into the orifices of the lateral sacs of the female, presumably to guide the secretion of the male accessory glands into the sacs. Shortly afterwards it is probable that the ovipositor is thrust more deeply into the genital pouch of the male so that the extremity of the phallosome is closely applied to the genital tubercle and the spermatozoa forced by the action of the ejaculatory sac into the spermathecae. Some of the spermatozoa remain in the spermathecae during the whole life of the female, probably nourished by the secretion of the cells surrounding them. The function and fate of the male accessory gland secretion, apparently passed into the female lateral sacs, have not been ascertained.

It is probable that during coitus the ovipositor is gripped dorsally by the lateral processes of sternum 5 and ventrally by the appendages of the anal

tergum. As little distension of the lateral sacs is possible, owing to the great thickness of their walls, there is little difficulty in separation, when the flies are disturbed.

Summary

In Part I the musculature of the internal organs of the male blow-fly, *C. erythrocephala*, testis, vas efferens, accessory gland, vas deferens, and ejaculatory sac and duct, is described, together with the movements observed in them and the passage of secretions down the system. It is suggested that the testicular and accessory gland secretions are ejaculated separately. The musculature, mechanics and co-ordinated movements of the external organs and associated structures are also described.

In Part II the generative organs of the female, except the ovaries, with special reference to their musculature and movements, and the musculature of the ovipositor are described and compared with those of *M. domestica*. It is suggested that the male accessory gland secretion is kept separate from the testicular secretion, the former passing into the lateral sacs of the female and the latter into the spermathecae.

In Part III the relation of the parts of the two sexes during coitus is discussed.

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STUDIES ON THE BIONOMICS OF *TRICHOSTRONGYLUS AXEI* (COBBOLD) AND ITS SEASONAL INCIDENCE ON IRRIGATED PASTURES

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(With 2 Figures in the Text)

Introduction

With the widespread adoption in California of ladino clover as a pasturage for sheep, especially for feeder lambs, the problem of controlling stomach worms has become increasingly important. The reason for this becomes quite obvious when one recognizes that under California conditions ladino clover requires very frequent irrigation and that the plant affords ample protection to the infective larvae of trichostrongyles. Heavy infections of *Trichostrongylus axei* (Cobbold) present a particularly acute clinical picture. The investigations described in this paper were carried on primarily for the purpose of determining the epidemiology of trichostrongylosis under conditions of irrigation.

Methods

On 27 August 1935, a band of twenty lambs was placed on a ladino clover pasture infected with *T. axei* and kept there for 12 months. This pasture was regularly irrigated every 10 or 12 days except during the rainy season. The lambs were all born in March and April of 1935 and had been exposed to *T. axei* ever since birth. Each animal had been treated twice with 2 oz. of a mixture of a 1 % solution of 40 % nicotine sulphate and 1 % copper sulphate. Two weeks intervened between treatments. These treatments were terminated just before the animals were weaned the first week in July. Despite the treatment the sheep were infected with *T. axei* at the beginning of the investigations. Faecal samples were taken by manual manipulation from each animal regularly every 2 weeks throughout the entire year of investigation and egg counts were made. Caldwell's method was used in making egg counts because it could be easily modified to accommodate small samples.

The Caldwell method was modified as follows:

(1) One gram of faeces is weighed out and placed in the bottom of a 30 c.c. shell vial calibrated at 10 c.c.

(2) Two c.c. of 30 % antiformin is added to the faeces in the shell vial and mixed thoroughly with a glass rod. The sample is then allowed to stand at room temperature for at least 1 hr.

(3) Sugar solution with a specific gravity of 1·23 is added to the 10 c.c. mark on the vial. The contents of the vial are thoroughly mixed.

(4) One-tenth c.c. of the prepared specimen is taken up with a pipette and spread on a slide to form a rectangular film.

(5) The eggs are counted with the aid of a mechanical stage under a 32 mm. objective and the number of eggs found is multiplied by 100 to express the number of eggs per gram of faeces.

Two slides of each specimen were prepared and counted for ova and the results were averaged to reduce error. The water content of each specimen was taken into consideration at the time of weighing in the laboratory; a relationship of 1 : 2 : 4 was considered to exist between the formed, unformed, and diarrhoeic faeces. The number of eggs present per gram of faeces was taken as an indicator of the magnitude of infection. It was determined by culturing faecal samples, that although parasites other than *T. axei* were present in the experimental animals they occurred in entirely negligible proportions.

In addition to the seasonal incidence survey, studies were conducted to determine various bionomic aspects of the immature stages. The techniques employed in these studies are described in the following section.

Discussion

Effects of temperature on eggs. A large faecal sample heavily laden with eggs of *T. axei* was divided into sixteen equal portions, placed in corked shell vials, and segregated into three groups as follows: five vials were held at room temperature as controls; five vials were placed in a constant temperature chamber at 38° C. with an atmospheric humidity of 29 % and were removed in regular rotation, one every 24 hr. beginning at the forty-eighth hour; and six vials were placed in a constant temperature chamber held at −6° C. and were removed in regular rotation, one every 24 hr. beginning after the ninety-sixth hour. The results were as follows:

Control group: The eggs hatched and many active larvae were found in four vials at the end of 48 hr. The fifth vial remained negative.

Group held at 38° C.: Active larvae were found in vials examined at 48, 72 and 96 hr. All other examinations were negative (the larvae having been killed, after hatching, by exposure to the heat).

Group held at −6° C.: Active larvae were found in vials, incubated at room temperature, after 69 hr. exposure to −6° C. All other examinations were negative (the eggs having been killed by exposure to the cold).

These results are not entirely in accordance with Mönnig (1930) who states that at 37° C. the embryonic development of *Trichostrongylus* spp. is completed very rapidly but that rarely does an egg hatch. However, the above results do agree with Mönnig's statement that at 37° C. those larvae which do emerge are soon killed. From the practical point of view, continuous exposure to temperatures of these magnitudes for periods exceeding those employed in the above tests would be very uncommon in stock-raising portions of Cali-

fornia. However, there are sections in the state where temperatures do considerably exceed 38° C. for a time each day over varying periods, and in such places a certain amount of lethal effect may be exercised on hatching larvae. In those regions of the world where temperatures of 38° C. or more may be sustained, one might expect a high larval mortality if there were not sufficient protection from such temperatures afforded by the cooling effects of evaporation, of irrigation or other water and transpiration of green plants. Where temperatures sufficiently low to be lethal obtain long enough to kill eggs there is usually a blanket of snow which affords a considerable degree of protection.

Effect of submergence in water on eggs. Faeces containing many *T. axei* eggs were broken up in water, strained through sieves to remove the larger particles, and transferred to a cylinder jar to settle. The supernatant fluid in this jar was decanted. Fifty c.c. of the sediment were divided equally between two Petri dishes and water was added to each to a depth of 15 mm. It was determined that there were approximately 160 eggs per c.c. of this resulting liquid. The Petri dishes were covered in such a way as to admit air and placed in diffused daylight at room temperature. This temperature attained a maximum of 28° C. and a minimum of 11° C. during the test period. After 5 days had elapsed samples of 1 c.c. each were removed daily from the Petri dishes and incubated in ova-free sheep faeces. A control sample was incubated at the beginning of the experiment and found to be positive. It was determined by this method that eggs submerged under water remained viable for 20 days, after which time all eggs were dead. No eggs hatched under water. Mönnig (1930) states that the eggs of *Trichostrongylus* do not develop in water deeper than about 3 cm. Theiler & Robertson (1915) report that eggs of *T. douglassii* remain viable under water for 2 months. It is not known whether the differences in survival noted above are due to differences in the depths of the water in which the eggs were placed or to different survival rates of the various species. Oxygen is necessary for the development of these eggs, and it is assumed that death is due to oxygen deficiency. Under natural conditions immersion in water for such periods as mentioned above is rare. However, in instances of prolonged floods it is possible that the egg population of a given place might be completely killed off. Furthermore, the amount of decaying material in standing water would determine the oxygen content of that water and might lower it to such an extent as to be lethal to *Trichostrongylus* eggs. Thus we have what may be at times a limiting factor in pasture and range infection.

Viability of eggs in dried faeces. Sheep faeces containing *T. axei* eggs were ground and dried to a light, dusty consistency and placed in a dish covered so as to permit access of air. This dish was placed in diffused daylight and kept at room temperature for a period of 10 months. Samples were taken from this source at weekly intervals and incubated. The cultures were positive, viable larvae being obtained up to 193 days after drying of the faeces, but on the 290th day and thereafter cultures were negative. Mönnig (1930) has

found that *Trichostrongylus* eggs containing complete embryos are resistant to desiccation, but eggs which are not completely embryonated never recover after the faeces have once been dried to a light, dusty condition. This author concludes that completely embryonated eggs can resist dryness for 15 months or longer. Theiler & Robertson (1915) state that *T. douglassii* eggs gave rise to the mature larvae after having been kept in dry faeces for 2 years. Both of these periods far exceed that observed in the present investigation. Zawadowsky *et al.* (1925*a*) reports that *Trichostrongylus* eggs do not resist desiccation. It is obvious that this latter author is in error. The discrepancies between the findings of Mönnig, Theiler & Robertson, and the present authors are difficult to explain unless they are due to differences in actual dryness determined by relative atmospheric humidity or to the different species of *Trichostrongylus* employed in the investigations. At any rate, it becomes apparent that pastures or ranges in California, heavily seeded with eggs, will not be sterilized by removing infected animals during the rainless season, since the eggs will survive desiccation for a period exceeding the dry months, unless exposure to direct sunlight would contribute additional harmful effects and such exposure would be very unlikely where forage is plentiful.

Effect of desiccation on non-infective larvae. Non-infective larvae of *T. axei* were placed on glass slides and dried for 30 min. at a temperature of 27° C. At the end of this time the larvae were covered with tap water. None of the larvae recovered. Mönnig (1930) determined that non-infective *Trichostrongylus* larvae survived drying in faeces for 7 days and had reached the infective stage. The drying was apparently done at room temperature under a forced draft of air. The same author dried non-infective larvae on glass slides, at an unreported temperature, and obtained no recoveries. It is apparent that completely embryonated eggs are much more resistant to desiccation than are non-infective larvae. The population of *T. axei* larvae may possibly be reduced in certain places by desiccation of the non-infective forms, but this is unlikely in view of Mönnig's (1930) findings which indicate that transformation to the infective stage occurs before non-infective larvae succumb to desiccation in faeces. Desiccation of non-infective larvae would not occur in irrigated pastures or in places, such as along the coast of the Pacific, where the atmospheric humidity is constantly high.

Effects of temperature on infective larvae. Infective *T. axei* larvae were taken from culture jars and separated into three groups.

Group I consisted of four vials in each of which were ten larvae. All of these larvae were immersed in tap water. Two vials were placed in a 38° C. chamber and two were placed in a −7° C. chamber. The larvae maintained at 38° C. lived for 408 hr.; the majority of these larvae had either shed or were beginning to shed their sheaths at the end of this period. The effect of this disensheathment upon the larvae or upon their infectivity is not known. The larvae maintained at −7° C. were still alive at the end of 48 hr., at which time the supply of material was exhausted. Mönnig (1930) reports that infective

Trichostrongylus larvae placed in water below 0° C. were alive at the end of 10 days of exposure.

Group II consisted of four vials in each of which were from ten to fifteen infective larvae in moist ova-free sheep faeces. Two vials were placed in a 38° C. chamber and two were placed in a −7° C. chamber. The larvae maintained at 38° C. remained viable for 192 hr.; however, they left the faeces on the first day and stayed in the water film on the side of the vials. The larvae maintained at −7° C. were still alive at the end of 48 hr., at which time the supply of material was exhausted. Mönnig (1930) reports that infective *Trichostrongylus* larvae live up to 12 days in moist sheep faeces at a temperature of 37° C., and at 0° C. are still alive at the end of 14 days.

Group III consisted of four vials containing dry, ova-free sheep faeces into each of which were placed twenty infective larvae. Two vials were placed in a 38° C. chamber and two were placed in a −7° C. chamber. At 24 hr. intervals a vial was removed from each of the chambers and the contents were moistened and incubated. The larvae kept in dry faeces at 38° C. were alive at the end of 24 hr. but dead at the end of 48 hr. The larvae kept at −7° C. were dead at the end of 24 hr.

Upon the exhaustion of the material in Groups I and II maintained at −7° C., infective *T. axei* larvae were placed on small squares of blotting paper, five larvae to the square. One group of ten such squares was placed in a −7° C. chamber and another group of ten squares was placed in a 38° C. chamber, where the relative atmospheric humidity was 29 %. The larvae maintained at −7° C. were alive at the end of 25 days but not at the end of 39 days; those maintained at 38° C. lived for more than 104 days.

From the above data it appears that infective larvae of *T. axei* possess considerable resistance to high temperatures. The resistance in water is greater than that in moist faeces. Since the larvae left the moist faeces to go into the water film on the sides of the vials moisture seems to be the determining factor. This idea is supported by the fact that larvae in dry faeces did not survive 48 hr. exposure to 38° C. However, the degree of moisture necessary for survival to exposure to high temperatures may not necessarily be great since larvae on blotting paper exposed to a temperature of 38° C. at an atmospheric humidity of 29 % were still alive at the end of 104 days. The species of *Trichostrongylus* with which Mönnig worked survived 4 days longer at a temperature of 37° C. than did *T. axei* at 38° C. This may have been due to the slightly lower temperature employed by Mönnig, to a difference in relative humidity, or to a difference in the species investigated. The resistance to heat manifested by *T. axei* appears to be sufficient to protect it against high temperatures encountered under natural conditions, even though suitable hosts are not readily accessible. Infective larvae of this species are less resistant to low than to high temperatures. It is difficult to estimate the effect of low temperatures on this stage of development under natural conditions. Where low temperatures prevail over considerable periods of time the usual mantle of snow gives the worms

some degree of protection. In all probability, where sheep are maintained in California during the winter months, the temperatures are not sufficiently low and prolonged to kill off the entire population of infective larvae. However, some of the larvae are undoubtedly killed in certain sections if hosts are not fairly readily available.

Effect of submergence in water on infective larvae. Infective *T. axei* larvae were removed from a culture jar and placed in a Syracuse watch-glass under 5 mm. of tap water and kept in diffused daylight at room temperature. All of the larvae were dead at the end of 231 days. Theiler & Robertson (1915) state that *T. douglassii* infective larvae remain viable in water for 13 months. Mönnig (1930) reports that infective *Trichostrongylus* larvae live for 7 months in 10 cm. of water at room temperature, for $3\frac{1}{2}$ months in 8 cm. of water over 5 cm. of mud in a fairly dark corner, and for $2\frac{1}{2}$ months in 8 cm. of water over 5 cm. of mud on a verandah where the sun could shine on them for a few hours each day. The differences in longevity as recorded above may be due to the differences in the depths of water in which the larvae were placed with consequent differences in the amount of oxygen available or to the different species of *Trichostrongylus* upon which the tests were made. Mönnig's work indicates that the larvae do not live as long in water containing mud as they do in clear water, and that they die off sooner in water exposed to sunlight than in water in the shade, even though there is not much difference in water temperature. It is obvious that the infective larvae population of a pasture is not disturbed by even prolonged submergence in water. Therefore, irrigation and even normal floods are not factors of any pasture sterilization value.

Effect of desiccation on infective larvae. T. axei infective larvae were segregated on glass slides, three larvae to the slide, and allowed to dry at room temperature, which ranged from 17 to 27° C., where the atmospheric humidity ranged from 35 to 51 %. All of the larvae were dead on the sixteenth day. Theiler & Robertson (1915) report that *T. douglassii* infective larvae resist desiccation for 9 months. Zawadowsky *et al.* (1925*b*) found that *T. instabilis* and *T. probolurus* revive after 2 months of dryness. Daubney (1928) states that *Trichostrongylus* infective larvae do not possess any great powers of resistance to desiccation. Mönnig (1930) reports that *Trichostrongylus* infective larvae dried on glass slides in the laboratory under conditions of rising temperature and dry atmosphere survived for 50 days; that of those dried and kept in a refrigerator at 0° C., 10 % recovered after 30 days; that of those dried and kept in the laboratory under conditions of falling temperature and moist atmosphere, 45 % recovered after 115 days; and that of those dried and kept in the laboratory under cold and dry conditions later followed by rising temperature and moist atmosphere, 0·1 % recovered after $8\frac{1}{2}$ months. The different results obtained in these various investigations may have been due to the different species investigated or to differences in factors which determine the actual degree of dryness, such as the presence or absence of debris around the larvae, the relative atmospheric humidity, and the temperature. Mönnig (1930)

further observed that the longer the larvae had been dry, the longer the time they required to revive and that frequently the larvae cast their sheaths when they revived. It is obvious that a cool, moist atmosphere affords the most favourable conditions and a warm, dry atmosphere very unfavourable ones. The warmer the air the more water vapour it can absorb and consequently the greater the actual degree of dryness. It would appear that in certain places ranges may be sterilized so far as infective larvae are concerned by exposure to such conditions of desiccation as occur under natural conditions. However, in irrigated pastures such conditions would never prevail. Furthermore, where the relative atmospheric humidity is high it is probable that the larvae can directly absorb moisture from the air through their sheaths and body walls.

Longevity of infective larvae in soil. Stender dishes (50 × 25 mm.) were about half-filled with fine (40 mesh) loam, and a glass tube extended through the soil to the bottom of the dish. Twenty *T. axei* infective larvae were placed on the surface of the soil in each dish. The soil was very moist at the beginning of the experiment and was kept moist from the bottom by means of pouring water through the glass tube. Longevity was checked by recovering larvae with the aid of a small Baermann apparatus. Active larvae were still being recovered at the end of 137 days. Mönnig (1930) reports that *Trichostrongylus* infective larvae placed in sheltered pots of clay soil, watered from the bottom, lived for 8½ months, and that in sandy soil they were dead after 5 months. This same author states that larvae in pots of clay soil watered from the top lived about 4 months. He believes that in this latter case the shorter life was probably due to the water washing the larvae into the soil and consequently forcing them to expend more energy in migration. Mönnig also states that larvae migrate more easily in sandy than in clay soil and die sooner in the former because of exhaustion. He further writes that a few weeks of dryness with summer heat suffices to kill larvae in clay soil which becomes very hot; also cracks appear which accelerate the drying process and larvae, being unable to migrate very well in such dense soil, are killed in the top layers. He believes that more larvae survive winter conditions in sandy than in clay soil because they can migrate deeper down for shelter in the former, but that, on the whole, the number of larvae that survive the winter is small. Mönnig reports that tests he has made show that continual very favourable conditions for migration cause rapid exhaustion and death of the larvae. In Mönnig's opinion the factors that cause soil sterilization are exhaustion of the infective larvae, which do not feed in this stage; desiccation; high temperature; exsheathment, which reduces resistance; and possibly the action of sunlight and of bacteria and other soil organisms. It is commonly believed that nematode larvae in soils live for a much shorter period in warm climates than in more temperate regions. It might be concluded from Mönnig's work that the type of soil plays an important role in determining the longevity of infective larvae in soil. Clay soil is less favourable than sandy soil under summer

conditions and more so under winter conditions. It appears from the above data that if suitable hosts of *T. axei* are kept off ranges or pastures for a year, or possibly 10 months or even less, under certain circumstances the land will be sterilized so far as this parasite is concerned, provided the infective larvae do customarily live in and migrate through the soil. The present authors observed very little migration in loam soil, and it is their belief that under normal conditions there is little reason to assume, in view of the bionomic and biologic necessities of the organism, that infective larvae of *T. axei* do reside or spend much time in soil; instead, it is believed that they spend their time on vegetation.

Phototropic responses of infective larvae. A glass tube 50 cm. long and with a diameter of 0·7 cm. was filled with tap water. In this tube were placed 200 *T. axei* infective larvae. The tube was then plugged at both ends and one-half of it was inserted into a black rubber tube, closed at one end. The apparatus was suspended horizontally over a table in strong diffused sunlight for 2 hr. No congregation of larvae was noted. In a second test all of the larvae were placed in the dark end of the tube and the apparatus was set up as before. No migration was observed. In a third test the situation was reversed by placing all of the larvae in the light end of the tube and again no migration occurred. Mönnig (1930) reports that his phototropic tests upon infective larvae of *Trichostrongylus*, the exact species of which is not given, showed that they migrate in a similar apparatus from both the very light and very dark ends of the tube to an intermediate zone just within the covered portion of the tube. The present authors finding no evidence whatsoever of phototropic reaction have come to the belief that the infective larvae migrate up vegetation because of a negative geotropic reaction. This behaviour may, of course, be conditioned at times by other external stimuli, such as temperature, humidity, and light intensity. Mönnig (1930) reports that *Trichostrongylus* larvae do not migrate upwards in a strong light but do in a diffused light.

Seasonal incidence of infection in lambs on irrigated pasture. The seasonal incidence of *T. axei* infection in the lambs kept for a year on an infected ladino clover pasture is shown in Fig. 1. The broken line shows the average infection for the entire flock of animals. The solid line shows the average infection for the entire flock with the exception of lamb no. 11, which had an unusually heavy worm burden and was considered to be exceptionally susceptible to *T. axei*. It is believed, therefore, that the solid line gives a more accurate picture of the condition of the flock than does the broken one which is distorted by the condition of a single animal.

It will be observed from Fig. 1 that there was, in general, a rise in the magnitude of the trichostrongylosis, as determined by egg counts, from the middle of August to the middle of January, when a peak was reached. After 17 January the infection rapidly dropped to an insignificant figure and remained so until the end of the investigation. No correlation could be detected between the degree of parasitism and the rate of weight gain. However, the infections,

with the exception of that of lamb no. 11, were subacute throughout the entire period of observation. The coldest period of the season occurred from 20 to 24 December, when the minimum temperature hovered between −4 and −2° C. The extreme ranges of minimum temperatures from the middle of October to 1 March was from −3 to 12° C. None of these temperatures is low enough to have a pronounced effect upon the pre-infective and infective larvae, nor were any of the low temperatures sufficiently prolonged to interfere appreciably with the development of the free-living stages of the parasite. Morgan & Oldham (1934) came to the conclusion that as regards infections with the smaller trichostrongyles, the winter months are the most critical

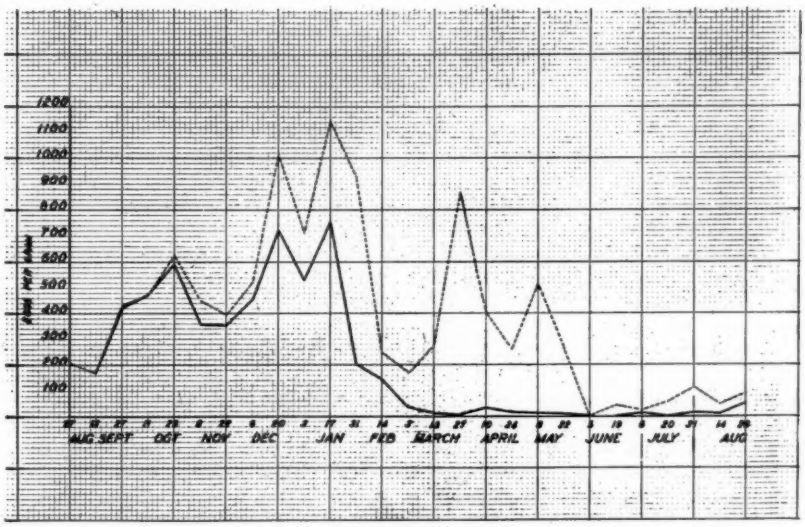

Fig. 1. Showing seasonal incidence of *T. axei* infestation in sheep on ladino clover pasture.

to sheep on heavily contaminated pastures and suggest as a possible explanation nutritional factors and adverse weather conditions. The peak of infection attained, during the investigation under consideration, on 16 January cannot be explained by the cold period from 20 to 24 December nor to nutritional factors because the egg counts had been increasing more or less steadily since the middle of August. It is believed that the irregularities in the generally ascending curve, shown in Fig. 1, from the middle of August to the middle of January can be explained as either experimental error or normal variation. Taylor (1934) considers a prolonged drought an important epidemiological factor because of the reduction in the quantity and quality of herbage which produces a condition of under-nourishment with consequently lowered resistance of the sheep to parasitic invasion. He also believes that a prolonged

grazing period necessitated by the lack of herbage during a drought may be concerned with outbreaks of trichostrongylosis. The same author states that the storing up of infective material in dried faecal pellets and the subsequent mass development of infective larvae during a period of moist, warm weather may also play an important part in the seasonal incidence of infestation. Drought and its effect upon vegetation did not influence the curve shown in Fig. 1 because the pasture upon which the animals were kept was regularly irrigated. For the same reason there was no prolonged grazing period. Warm weather may possibly have been a factor in the present instance, especially if one takes into consideration the constant build-up in the free-living population, but it might well be expected in such an event that the peak would be

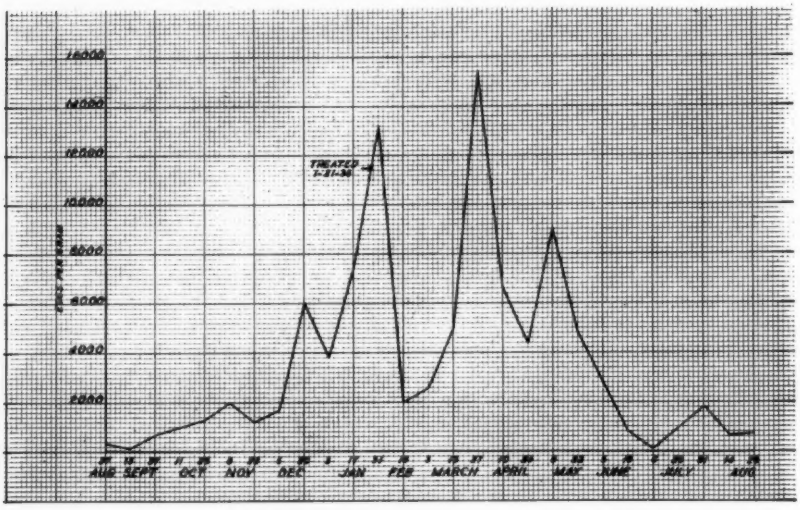

Fig. 2. Showing seasonal incidence of *T. axei* infestation in a particularly susceptible sheep and the effect of treatment with 1 % copper sulphate solution.

attained earlier in the season. Age resistance, or acquired resistance, or both are the only apparent factors which cannot be refuted in an attempted explanation of the seasonal incidence curve. Consequently, it may be inferred that the seasonal incidence of *T. axei* infestation is determined under irrigated pasture conditions in California by acquired, or age resistance, or both and not by environmental influences upon the free-living stages.

Effect of treatment upon T. axei *infection.* Fig. 2 shows the course of *T. axei* infection in a particularly susceptible animal, lamb no. 11. This animal was treated on 21 January with 50 c.c. of a 1 % solution of copper sulphate. Ten days later a peak was attained. During the next 2 weeks a marked decline in the number of eggs per gram of faeces occurred. However, by

27 March a new peak was reached which exceeded that of 31 January by 2200 eggs per gram of faeces. The curve rapidly dropped a second time, but on 8 May another, but much lower, peak occurred.

In another experiment 300 lambs very heavily infected with *T. axei* were divided into three groups of 100 lambs each. Group A was treated with 50 c.c. of a 1 % solution of copper sulphate per animal; Group B was treated with 50 c.c. of a mixture of 1 % copper sulphate and a 1 % solution of 40 % nicotine sulphate; and Group C was left untreated as a control. All of these animals were treated on 16 March and allowed to graze together. Faecal samples were collected and egg counts and faecal cultures were made from each of the three groups on 14 March, 30 March and 20 May. From these data it was determined that there was a 63 % decrease in *T. axei* in those animals treated with the copper sulphate solution; a 96 % decrease in those treated with the mixture of copper sulphate and nicotine sulphate; and a 24 % decrease in the control animals. It appears, therefore, that the mixture of copper sulphate and nicotine sulphate is a more efficient anthelmintic against *T. axei* infestations than is a 1 % copper sulphate solution.

In still another test 200 lambs heavily infected with *T. axei* were left untreated and kept on the same pasture, and under the same conditions, where the infection occurred. These lambs manifested acute clinical symptoms of trichostrongylosis and deaths were occurring. In 3 weeks time after the first observations were made a spontaneous recovery, so far as clinical symptoms were concerned, had been effected. Several other instances of the same sort of recovery were discovered through questioning various sheep ranchers.

In several instances lambs pastured on ladino clover and manifesting acute clinical symptoms of trichostrongylosis have been given access to all the hay they wanted regularly every 2 weeks as they were moved from one pasture to another. They ate ravenously of this hay for 2 or 3 days each time. Where this practice has been followed and continued all symptoms of trichostrongylosis have disappeared without the administration of any drug or any other change in the management of the animals. It appears, therefore, that the addition of dry feed to a diet of green vegetation *may* be sufficient to control *T. axei* infestations in sheep on irrigated ladino clover pastures. Controlled experiments must be conducted, however, before a definite statement can be made relative to the therapeutic value of dry feed.

From the data discussed above certain general conclusions concerning natural sterilization of ranges and pastures may be drawn. It must be borne in mind, however, that such conclusions are of necessity very general, since meteorological factors vary considerably in any given place and since the free-living stages of a parasite such as *T. axei* are influenced more by micro-climate than by macro-climate and micro-climatics is at the best but little understood. It is concluded that temperatures sufficiently high and prolonged to exercise *per se* a lethal effect upon eggs and infective larvae are rarely encountered in any of the sheep-raising sections of the United States. In irrigated

pastures the cooling influences of evaporation of irrigation water and transpiration of green vegetation are probably sufficient to protect the developmental stages of the parasite from high temperatures.

Non-infective larvae are not affected by high temperatures encountered under natural conditions because they develop too quickly to the infective stage. Temperatures sufficiently low to kill eggs and infective larvae occur commonly under natural conditions in certain parts of the United States, but in such places there is usually a heavy blanket of snow which may afford enough protection, so that a large percentage of the eggs and larvae survive. Desiccation, which is determined by temperature and atmospheric humidity as well as by standing water, may eliminate the infective larval population from ranges, but not from irrigated pastures, if sufficiently prolonged. Fatal desiccation of eggs in faeces seems very unlikely, but it is possible that direct exposure of the eggs to the sun might cause death. Non-infective larvae may be killed on dry ranges where the desiccation rate is high, but where there is even a small amount of protection against the desiccating influences the more resistant infective stage will be reached before lethal effects are obtained. Submergence in water, unless the floods are unusually prolonged or a great deal of decaying vegetation is present, does not play a role in inhibiting the build-up of a *T. axei* population on a pasture or range; it is not an inhibitory factor on irrigated pastures.

Summary

1. *T. axei* eggs in sheep faeces hatch at a temperature of 38° C. and a relative atmospheric humidity of 29 %, but all of the larvae are dead 96 hr. after the initiation of the exposure. Eggs kept in faeces at −7° C. are killed within 96 hr.

2. Eggs do not hatch while under water but remain viable for 20 days under 15 mm. of water at temperatures ranging from 11 to 28° C. Under natural conditions the longevity would in all probability be influenced by such factors as depth of water, amount of decomposing organic debris, etc., which determine the amount of available oxygen present.

3. Completely embryonated eggs in sheep faeces possess a high resistance to drying. It is apparent that ranges and pastures in California cannot be freed of eggs by keeping suitable hosts off them during a single dry season, because the eggs can survive desiccation for a period exceeding the dry season. It is possible, however, that if the eggs were directly exposed to the rays of the sun they might be killed.

4. The non-infective larvae of *T. axei* are very much less resistant to desiccation than are the eggs. The population of non-infective larvae would, however, under the more common natural conditions, normally attain the infective stage before succumbing to desiccation.

5. Infective larvae lived for more than 104 days at a temperature of 38° C. and a relative atmospheric humidity of 29 %. At −7° C. they were alive at

the end of 25 days but were dead on the thirty-ninth day. It is believed that under natural conditions such larvae would not be exterminated by high temperatures. At low temperatures the usual covering of snow might be sufficiently protective to prevent death.

6. *T. axei* infective larvae survived 230 days' submergence in 5 mm. of water at room temperature in diffused daylight. Direct sunlight appears to have some lethal effect. The amount of oxygen available in the water appears to determine to a considerable degree the longevity of the larvae. Under natural conditions infective larvae can survive even greatly prolonged flooding.

7. The survival of infective larvae to desiccation is dependent upon the actual degree of dryness attained. Larvae dried on glass slides at room temperature ranging from 17 to 27° C. with a relative atmospheric humidity of from 35 to 51 % survived up to 16 days after the initiation of exposure.

8. Different investigators have obtained different results in studying the longevity of infective larvae in soil. The writers believe that the infective larvae do not spend much time on soil or migrate through it unless to escape some unfavourable environmental factor such as particularly high or low temperatures or desiccation, or unless they are accidentally washed into it during heavy rainfall.

9. No evidence of phototropic behaviour was observed. It is believed that vertical migration up vegetation is caused by a negatively geotropic reaction which may be conditioned or altered at times by stronger external stimuli resulting from extreme temperatures, bright sunlight, low atmospheric humidity, etc.

10. It is believed that the seasonal incidence of trichostrongylosis on irrigated ladino clover pastures in California is determined by acquired resistance, age resistance, or both.

11. A mixture of a 1 % solution of 40 % nicotine sulphate and 1 % copper sulphate appears to be a more efficient anthelmintic against *T. axei* than a straight 1 % copper sulphate solution. Changing from green feed to hay or rendering hay available along with green feed may possibly have a therapeutic effect. Spontaneous recovery, so far as clinical symptoms are concerned, from an acute trichostrongylosis, without the administration of a drug or changing the diet, seems to be common, if not the rule, in the majority of lambs in an infected band.

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THE DEVELOPMENT OF *MONIEZIA EXPANSA* IN THE INTERMEDIATE HOST

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(With Plate XVI, containing Figs. 1–9)

Introduction

The life histories of the taenioid tapeworms of the dog, cat, and man have been known for three-quarters of a century. Since that time the life cycles of other species have been discovered, and in recent years the number has been much increased. Indeed, the course of development has been traced in representatives of most of the larger groups of cestodes. With rare exceptions at least two host species are required, one of which harbours the adult and the other the larval stage of the parasite. In almost all known life histories the larva, after developing to an infective stage in a suitable intermediate host, is accidentally ingested by the final host with food, and sexually mature cestodes are usually found in the intestine of carnivorous vertebrates.

An apparent exception to the general rule obtains in the family Anoplocephalidae. These cestodes are very numerous, world wide in distribution, and occur frequently in the herbivorous domestic animals. Although repeated attempts have been made for over fifty years to discover the life cycle of these tapeworms, it has remained until recently one of the major problems of parasitology. A review of the family and the life-history studies done on its members was made by Stunkard (1934). In that paper he reported experiments carried on for over a year to determine how the tapeworms of rabbits complete their life cycle. An intensive study was made of the eggs and onchospheres. Possible methods of development were outlined and arranged under two headings, those which postulate direct development and those which predicate the necessity of a developmental phase in an intermediate host. The statement was made that if only one host is required, the onchospheres must be infective for that species and the postulate is subject to direct experimental test. A series of carefully planned and controlled experiments demonstrated that rabbits could not be infected by ingestion of *Cittotaenia* eggs, and that direct infection with species of that genus is impossible. From these studies it was concluded, "that an intermediate host is required; that the onchospheres when voided are infective for the intermediate host; and that the larvae develop to the cysticercoid stage in the body cavity of some invertebrate animal". Stunkard declared that the information then available was sufficient to indicate the characteristics of the intermediate hosts of anoplocephaline

cestodes with considerable assurance. He listed the bionomic features of these hosts and stated: "The number of invertebrate animals which satisfy all of these requirements is small enough that the discovery of the life cycle may confidently be expected."

Honess (1935) infected the domestic rabbit by feeding grass from an area frequented by wild rabbits. According to him, the volume of the scolex as it enters the rabbit was 31·9 times greater than the egg and 1306 times greater than that of the onchosphere. He concluded: "From this increase in mass and the change in structure from the hexacanth embryo to the adult scolex, it is evident that the change must take place in a nutrient medium permitting growth, and therefore in an intermediate host."

In a series of papers, Stoll (1935*a*, 1935*b*, 1936, 1937*a*, 1937*b*) reported studies on the epidemiology of *Moniezia* infection in sheep and cattle. In the first paper he stated: "The demonstrated failure of *Moniezia* to spread from infested to uninfested areas except within infected sheep tends to support the conception, not of an intermediate host, but of direct infection from contaminated soil or forage." The last paper reports the failure to recover tapeworms from the intestine of lambs earlier than 9 days after initial exposure on infected pasture. This failure and the characteristic immunity which develops in sheep against *M. expansa*, led to the postulate of a preliminary, tissue-invasive stage for this cestode in sheep.

In preliminary accounts, Stunkard (1937*a*, 1937*b*) reported experiments in which various minute, terrestrial invertebrates, chiefly insects, had been used to determine the life history of *Cittotaenia variabilis* from rabbits and *Moniezia expansa* from sheep. Eggs of these cestodes were fed in the spring of 1936 to tyroglyphid mites and the onchospheres of both species were recovered from the body cavity. Eggs of both species were fed also to mites of the genus *Galumna*, and again the larvae emerged and migrated to the body cavity. Thousands of galumnid mites were exposed to eggs of *M. expansa*, and specimens dissected at various intervals yielded a series of developmental stages of the cestode larvae, from the onchosphere to the mature cysticercoid. In one instance, 200 mites exposed four months to *Moniezia* eggs, were fed to a lamb which became infected.

The studies have been continued and it appears desirable to make a more complete report and present the evidence on which the earlier accounts were based.

Material and methods

Cestode eggs have been obtained chiefly from two sources, the markets and the field. During the winter, when rabbits are available in the shops, gravid proglottids of *Cittotaenia* are easily obtained. Such material has the advantage that the species can be determined with certainty, and although most of the worms were *C. variabilis*, *C. perplexa* does appear occasionally. This material has a distinct disadvantage, since gravid segments often contain

onchospheres which, although apparently mature, are not infective. Many experiments have failed because the onchospheres were not fully mature. During the closed season, rabbit faeces have been collected in the field. It is necessary to take only fresh pellets, since old and weathered ones yield very few cestode eggs. In certain areas the wild rabbits are heavily infected and eggs may be obtained from faeces, whereas in other districts no infection of rabbits has been found. It is impossible to make specific determination of eggs from faeces, and results of such collection are frequently irregular and uncertain. *Moniezia* eggs may be obtained throughout the year by collecting tapeworms at a slaughter-house. Since long pieces of a strobila may be picked up, specific identification is not difficult. Only eggs from gravid proglottids of *M. expansa* were used. It is important that the onchospheres be fully mature, otherwise they are not infective.

Previous studies had demonstrated that treatment with enzymes and passage through the digestive tract of various animals would not induce hatching of eggs, whereas mechanical injury to the shell was followed by emergence of the larvae in a large percentage of cases. These observations suggested some arthropod, either a mandibulate species or one provided with a grinding stomach, as the host of the larval stages. From epidemiologic and ecologic considerations, as outlined by Stunkard (1934), it appeared likely that small ants might serve as intermediate hosts of anoplocephaline cestodes. During the summer of 1932, thousands of specimens were collected near Hamburg, Germany, from localities where the wild rabbits were heavily infected. These ants were dissected under a binocular microscope in the search for cysticercoids of a natural infection, and all of the material at the end of a dissection was fed to tame rabbits, as a check against larvae which might have been overlooked. Although these attempts were futile, the study was continued in New York. When natural infections could not be found, ant colonies were brought into the laboratory, maintained in plaster of paris nests designed by Dr W. S. Creighton, and exposed to cestode eggs. The announcement by Jones & Horsfall (1935) that *Tetramorium caespitum* and *Pheidole* sp. serve as intermediate hosts of two species of *Raillietina* increased the suspicion that ants harbour the larval stages of anoplocephaline cestodes. In fact, at that time I had colonies of both these ants in the laboratory. But all attempts to infect ants proved negative, and the next spring the ant experiments were discontinued.

A fortunate combination of circumstances led to the discovery of the intermediate host of *Moniezia expansa*. For several years Drs Ludwig and Fox of this laboratory have conducted experiments with Japanese beetle larvae and their cultures were often overrun with tyroglyphid mites, probably *Rhizoglyphus* sp. Since these mites are scavengers and live in the upper layers of the soil, they were fed in February 1936 eggs of both *Cittotaenia* and *Moniezia*. When dissected a few days later, onchospheres of both cestodes were recovered from the body cavity. Although the structure and habits of these mites did

not favour the hypothesis that they were suitable intermediate hosts of *Cittotaenia* or *Moniezia*, and the onchospheres did not develop in them, the fact that the larvae emerged from the eggs and migrated to the body cavity indicated that the natural host was to be sought in some related species. Accordingly, other free-living mites were collected and exposed to infection.

The oribatid mites appeared to possess the requisite characteristics for intermediate hosts of anoplocephaline cestodes and they proved susceptible to infection by onchospheres of both *Cittotaenia* and *Moniezia*. Thousands of these mites, collected from areas where sheep had not been pastured for many years, have been brought to the laboratory and used in infection experiments. Since it was sometimes difficult to obtain *Cittotaenia* eggs, and eggs of *Moniezia* were readily available, the latter were used in most of the work. The experiment was conducted in two ways; after exposure to cestode eggs, mites were dissected at intervals to trace the development of the larvae, and one group of 200 was maintained for more than 4 months, when it was fed to a lamb. Dissection of mites at various intervals after exposure showed much development of the larvae, but, as a result of high mortality among the mites and the use of immature cestode eggs in some of the experiments, no mature cysticercoids were obtained in the dissections of the first year. The lamb became infected, but this result is not regarded as convincing, since accidental infection was not precluded.

In later experiments, the use of specially designed nests reduced mortality of the mites and care was taken to use only eggs which contained active larvae. In the summer of 1937, a series of developmental stages from the onchosphere to the fully formed cysticercoid was recovered from the body cavities of mites and the life cycle was announced (Stunkard, 1937*a*, 1937*b*). With the improved nests it has been possible to rear mites from eggs to maturity, and such laboratory-raised specimens are available for experimental use.

The specific identity of the mites which served as intermediate hosts of *Moniezia expansa* is still undecided. The taxonomy of these minute animals is in a very unsatisfactory condition, and authorities differ whether particular features are of generic or specific importance. All of the mites used fall within the generic concept of *Galumna* as stated by Ewing (1935). The contents of one nest, however, were submitted to Dr A. P. Jacot for identification, and in the group he found *Ceratozetes gracilis*, *Galumna longipluma*, *Zetes elimatus*, *Z. emarginatus*, *Z. emarginatus coscobensis*, and *Z. nervosus*.

It is to be noted that information concerning the life history, food habits, and general biology of these mites is very fragmentary. Referring to the oribatid or beetle mites, Jacot (1932) stated that they are active chiefly at night when they come swarming out of innumerable crannies to find bits of frass, the tiny mushrooms of moulds and mildews, algae, dead plant parts and other organic matter. They are generally eyeless, but have tactile and ambulatory senses well developed. They rarely walk faster than an inch a minute, and some move so slowly that their progress is barely discernible. Although primarily

inhabitants of the forest floor, they have radiated into various habitats. They constitute the outstanding faunal element of mosses. Some ascend trees and hide by day under the bark, especially on the north side, browsing on the green algal film which develops after every rain. Some ascend pasture and meadow grasses every evening and descend at dawn, passing the day in the shelter of the sod. They are abundant in sod, on pasture dung, on dead branches, twigs and logs of the woodland floor as well as in the earth. Although distributed from the arctic to extreme southern regions they are most abundant in moist, temperate woodland where every cubic foot of humus and surface litter contains thousands of individuals. He postulated that the state of Massachusetts contains 80 to 100, and the New England area about 200, species. Referring to the genus *Zetes*, Jacot (1935) declared that it is found throughout the world, except in the northernmost regions.

OBSERVATIONS

The onchospheres of *Moniezia* do not differ essentially from those of *Cittotaenia* (Fig. 1) as described by Stunkard (1934). At emergence, the larva measures about 0·02 mm. in diameter. It consists primarily of parenchymatous cells, many of them differentiated into muscular elements, some of which comprise the body wall and others serve to move the hooks. In the interior there are from six to twelve larger cells, recognized in *Diphyllobothrium* by Vogel (1929, 1930) as embryonic or germinal in nature. When the larvae emerge from the eggs they are very active and almost immediately penetrate the wall of the digestive tract to reach the body cavity. The location in the mite where hatching actually occurs has not been determined, since the onchospheres have been found only in the body cavity.

The rate of development in the mite is variable and such differences have been reported also for other species by Joyeux (1920) and Venard (1938). Joyeux stated (p. 98): "Il y a donc de grandes variations de vitesse dans l'évolution, suivant les saisons, la température, l'hôte et probablement aussi d'autres facteurs qui nous échappent." Larvae recovered a week to 10 days after exposure may be only 0·022–0·024 mm. in diameter, whereas one removed on the fourth day measured 0·025 by 0·03 mm. With the method employed, it is impossible to determine when a mite has eaten the cestode eggs, and several days may have elapsed between exposure and ingestion of eggs. In the body cavity of the mite the onchospheres are motile for several days, and one removed 2 weeks after exposure (Fig. 2) was very active, as may be observed by examination of the photograph. The hooks moved during the 10 sec. exposure, and some of them appear double.

As development proceeds, the mesenchymal cells of the onchosphere degenerate and the muscles atrophy. As a result, the hooks become functionless, lose their regular positions, and may be extruded. Meanwhile the germinal cells multiply and decrease in size. With these changes the form of the onchosphere is lost and the larva becomes spherical and immobile (Figs. 3, 4). In

fact, the cells which composed the onchosphere have largely disappeared, and the new larva is built up of cells derived from the germinal ones of the onchosphere. At the end of 4 weeks the larvae are 0·06–0·09 mm. in diameter and by 6 weeks they may measure 0·07–0·1 mm. At this time they begin to elongate and are wider at one end than the other. In the body of the larva a cavity appears and gradually shifts towards the narrower end where the hooks are located. In front of the cavity, where the scolex will develop, there is rapid proliferation of cells. At about 8 weeks the larvae measure 0·08–0·13 mm. in length and 0·06–0·1 mm. in width. Calcareous bodies have begun to appear in the parenchyma and the cells in the peripheral portion differentiate to form the longitudinal and circular muscles of the body wall. The cuticula which appeared earlier as a thin, structureless membrane becomes considerably thickened and the larvae are not so fragile as formerly. At about 10 weeks, the body walls of some of the larvae are sufficiently well developed so that they become mobile and slowly elongate and contract. They are oval to pyriform in shape and the caudal portion begins to assume the appearance of a cercomere. By this time the outlines of the suckers may be recognized as condensations of cells. There is very little further growth, but differentiation of the tissues produces a vermiform larva which may extend to a length of 0·3 mm. At the end of 15–16 weeks the invagination of the cephalic end has been accomplished and the cysticercoid is fully formed. It is spherical, about 0·18 mm. in diameter, with a small fibrous appendage which represents the remains of the atrophied cercomere.

The course of development may be illustrated by the records of two infection experiments:

Experiment I. 170 mites collected from Bronx Park, New York; *Moniezia* eggs from a gravid proglottid were added 29 April and 8 May 1937.

13 May. Nine mites dissected, one onchosphere 20μ in diameter recovered.

14 May. Five mites dissected, one onchosphere 31 by 25μ recovered; photographed alive (Fig. 2).

27 May. Five mites dissected; two larvae were found in one mite. One was slightly injured in the dissection but they were photographed (Figs. 3, 4). The larger one, which is clear of the shell, measured 93 by 83μ.

Experiment II. Eighty mites collected at Woods Hole, Massachusetts, in January 1937, maintained in the laboratory since that time, were exposed on the same dates and with eggs from the same source as those of Exp. I.

18 June. Seven mites dissected; a single larva which contained 6 hooks and measured 90 by 80μ was recovered.

29 June. Six mites dissected; a single larva which contained 6 hooks and measured 132 by 96μ was recovered.

30 June. Five mites dissected; two larvae were found in one mite. Each contained 6 hooks; one measured 145 by 138μ and the other 34 by 30μ.

7 July. Five mites dissected; one mite contained a single larva; it was mobile and contraction of circular muscles gave the appearance of three seg-

ments. Alive it measured 288μ in length; the cephalic portion was 122, the middle portion was 72, and the caudal one in which three hooks were visible measured 43μ in width. This larva was studied for some time and had begun to blister when it was photographed (Fig. 6). In another mite, a larva almost as large as the one just described was found, together with a much smaller one.

10 July. Seven mites dissected; one contained a larva, with 6 hooks, which measured 114 by 110μ (shown in Fig. 5); another mite contained a larva which measured 317 by 117μ; while a third contained two small larvae which measured 86 by 74, and 62 by 55μ respectively.

12 August. Four mites dissected; one mite contained two larvae. The first measured 95 by 55μ; it moved actively for about ten minutes, a definite cercomere was present and three hooks were visible. The second larva measured 131 by 69μ; it was slightly injured at the caudal end and no hooks were seen. Both of these larvae were fixed, stained and mounted.

17 August. Five mites were dissected; one mite contained two fully formed cysticercoids. One of them, shown in Fig. 7, measured 0·17 mm. in diameter. The other, shown in Figs. 8 and 9, measured 0·18 mm. in diameter. It contained a clearly visible and moving scolex. The cyst wall was about 5μ in thickness, the scolex was 110μ in diameter, and the suckers measured 45–50μ. In Fig. 8, the larva was oriented in such a way that the attachment of the cercomere appears in the photograph. Fixed, stained and mounted, these larvae measure 0·149 and 0·158 mm. in diameter.

DISCUSSION

The observations here recorded, tracing the development of *M. expansa* in the intermediate host, demonstrate the necessity for such a stage in the life cycle and discredit the hypothesis of direct infection. Since one species in the family Anoplocephalidae develops its cysticercoid in free-living mites, it is probable that others follow the same course. The discovery of the life cycle of *M. expansa* confirms the prediction of Stunkard (1934) concerning the bionomic features of the organisms which harbour the larval stages of these cestodes.

An observation of Hall (1934) acquires added significance in the light of present information. While studying the life history of *Thysanosoma actinioides* in 1912, he noted, "that the terminal segments of this worm never show shelled eggs, that detached segments in the cecum and colon always showed shelled eggs, that segments in freshly passed manure usually, but not always, have eggs in them, and that segments in manure which has been passed for some time seldom have eggs in them. When a fresh proglottid was placed in a dry bottle, left there for a time, and then examined, the segments would be found devoid of eggs and there would be a ring of eggs at some distance around the segment, the eggs apparently being ejected from the segment as it dried." From this observation he concluded: "The significance of this distribution of

eggs by discharge from the segment may be more evident when the life history of this worm is known. At the moment it looks like evidence that a coprophagus insect is not an intermediate host, since it would seem to be of little advantage to scatter the eggs from a segment lying on the surface of manure when most of them would then land on vegetation or soil away from the manure." The observation reported by Hall has been noted repeatedly in the present study. When gravid segments of *Moniezia* or *Cittotaenia* have been left for several days in a Petri dish, the eggs which they originally contained are found disposed in a layer some distance from the segments. Stunkard (1934) stated: "Ordinarily the eggs are very abundant in the faeces and if the intermediate host were to pick them up and feed on them, it would probably be unable to withstand the severity of the infection. The ingestion of the onchospheres therefore is almost certainly accidental or incidental in the feeding habits of the intermediate host." Since the mites are not primarily coprophagus, but live among the roots of grass, the distribution of eggs as recorded by Hall would increase the probability of their ingestion and prevent a mass infection which might result in the death of the intermediate host. The distribution of eggs would be facilitated by rain, and eggs washed into the upper layers of moist soil would remain viable for long periods of time.

Whether heavy infection of mites results in their death is problematical. The mites are very small and their eggs are relatively large, so the internal organs are capable of withstanding much pressure. Four is the largest number of larvae found in a mite and ordinarily when more than one is present, they are correspondingly smaller than in cases of single infection. In multiple infection, the development of the parasites appears to be retarded, and they mature at a smaller size. Not infrequently one large and two or three smaller ones have been found. The earlier or more rapid development of one larva may retard or inhibit the development of others. Similar results have been recorded for other cestodes and the phenomenon appears to be a general one.

The data presented by Stoll (1937*b*) are very interesting, but it is doubtful to what extent they support his hypothesis of direct infection and a preliminary tissue invasive stage of *M. expansa* in sheep. Direct infection by *Hymenolepis fraterna* is well established, and Joyeux (1924) reported a similar life cycle in *Cylindrotaenia*. In these instances, the onchospheres emerge from the eggs in the intestine of the final host and penetrate the gut wall where they transform into cysticercoids. With further growth, they are liberated into the lumen of the intestine and develop to sexual maturity. In *Hymenolepis*, at least, the cysticercoid may develop either in an invertebrate animal or in the intestinal wall, and it seems probable that the latter method is a recent modification of the more general one in which an intermediate host is required. Numerous investigators in the past fifty years have attempted without success to infect animals with eggs of their anoplocephaline cestodes and it has been demonstrated repeatedly that the eggs of these cestodes are not infective for their definitive hosts.

Stoll (1937 *b*) referred to the stage which supposedly invades the tissue as (p. 148) "an embryo still incapable of intestinal residence", and (p. 156) as "the infective agent". His paper however contains no statement concerning (1) the nature of this "infective agent", (2) its location before ingestion by sheep, or (3) the tissue invaded. In all known life cycles of cyclophyllidean cestodes, the parasite is transferred from one host to another in one of two larval stages. The first larval stage is the onchosphere and it develops within the egg. It is always liberated in the intestine of the next host, is provided with hooks for penetration, and always invades the tissue. The second larval stage is the cysticercoid or cysticercus and this larva seldom, if ever, leaves the intestine. It consists essentially of the scolex of the cestode and at the opposite end a region which contains the excretory pore and which becomes the future terminal segment of the tapeworm. Behind the scolex there are germinal cells which multiply and later produce the proglottids. The scolex of the cysticercoid of *Moniezia* is unarmed, very delicate, and probably incapable of tissue invasion. Although the statement of Stoll is very indefinite, his hypothesis, of an "infective agent" which is "incapable of intestinal residence" and which invades the tissue, would seem to imply that infection of sheep is accomplished by the onchosphere. Since repeated attempts to infect sheep by the ingestion of eggs have given only negative results, the hypothesis appears to be contrary to all experience.

In his search for small cestodes in the intestine of sheep, Stoll washed the opened gut wall under a shower head of warm water over nested screens of 20, 40 and 60 meshes to the inch. Although the size of the wire is not given, and a heavy wire would reduce the size of the openings, it is apparent that young worms would readily pass through the two coarser screens and even if the wire occupied one-half of the surface, a larva 110–200μ in diameter would pass the finest screen. Moreover, the young worms are delicate and pliant, soft and easily compressed; in a current of warm water they would probably elongate and consequently become smaller in diameter. The method utilized by Stoll, therefore, would almost certainly allow the smaller worms to pass with the water washing the tissue, and make it impossible to recover them. The smallest specimens recovered by Stoll measured approximately 1 cm. in length and, if a relatively constant rate of growth is maintained, about 1 week would be required for the cysticercoid to attain that size; which agrees with the period after infection in which Stoll failed to find the young worms. Accordingly, the failure to find the worms may be explained by the technique employed.

The fact that sheep develop immunity to infection by *Moniezia* was regarded by Stoll as evidence for a tissue-invasive stage of the cestode. Reference was made to experiments by Seddon (1931) to determine the nature of this immunity. Stoll noted a difference in degree of immunity to two species of *Moniezia*, an observation which agrees with the report of Stunkard (1932) that European hares and rabbits manifest a differential degree of resistance

to superinfection by different species of *Cittotaenia*. In his paper on the superinfection of cats with *Taenia taeniaformis*, Miller (1932) stated: "The immunity of mammals to superinfection with adult stages of cestodes has not been extensively investigated by the experimental method. The published data on this subject consist chiefly of reports of the finding of individuals of two or more species, or even genera, of tapeworms coexisting in the intestine of a mammal. The experimental work has been done largely with species of the genus *Hymenolepis* in rats. The data are conflicting, and some of the experiments lacked controls." Knowledge of the immunity developed by animals against their cestode parasites is fragmentary. Probably all helminth parasites induce the development of some degree of immunity on the part of their hosts, although the amount and nature of the resistance may vary greatly. For certain species, especially tissue parasites, the immunity may be humoral and general, whereas in other instances the immunity may be local. In the case of *Moniezia*, Seddon's experiments suggest a local and intestinal, rather than a general immunity. An emulsion of *Moniezia* material, injected subcutaneously, did not protect against subsequent infection, whereas another sheep, given a suspension of this material orally, did not become infected. If the absorption of *Moniezia* material by the intestinal wall will produce immunity, no tissue-invasive stage is required.

Summary

When eggs of *Monieza expansa* are fed to galumnid mites, the onchospheres emerge in the intestine and migrate to the body cavity. Here they undergo metamorphosis and develop into cysticercoids. The dissection of mites, at various intervals after exposure, has yielded a successive series of developmental stages. Protocols of the experiments and photographs of living larvae are presented. These results demonstrate that mites serve as intermediate hosts of *Moniezia*, and probably also of other anoplocephaline cestodes.

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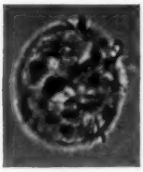
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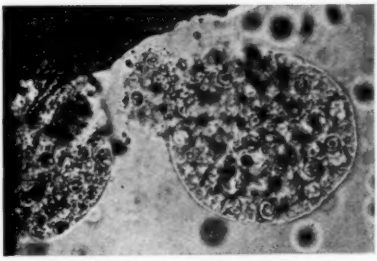
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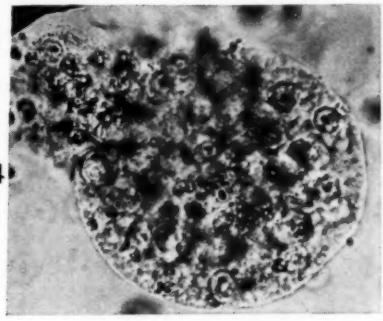
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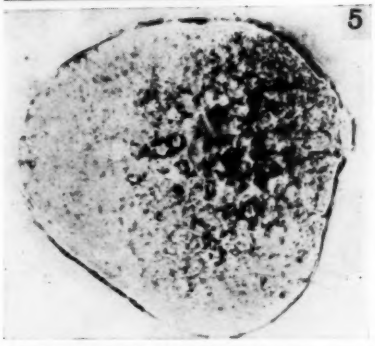
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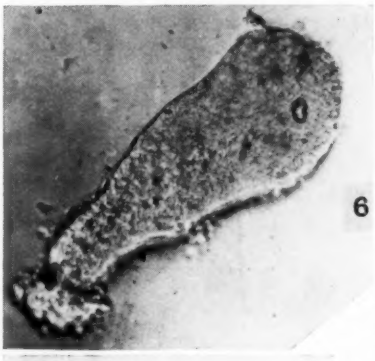
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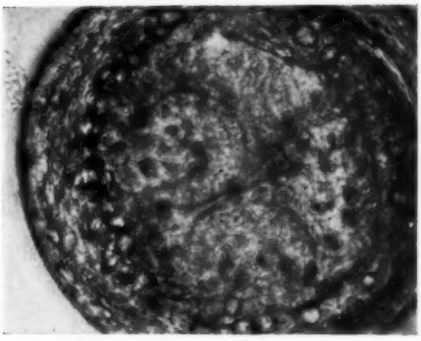
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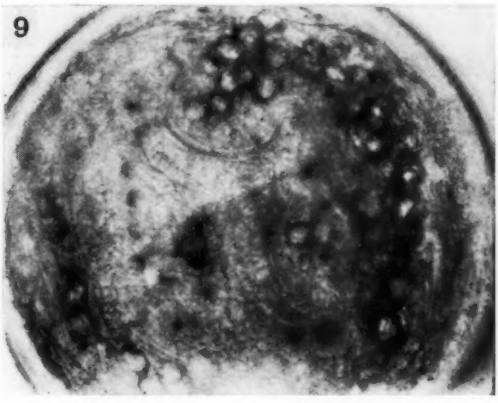
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EXPLANATION OF PLATE XVI

These figures are unretouched photographs of living larvae. Those shown in Figs. 2–9 were removed from mites fed eggs of *Moniezia expansa* on 29 April and 8 May 1937. The date of dissection and size of larva are given.

Fig. 1. *Cittotaenia variabilis*, onchosphere in pyriform apparatus, after removal from shell; larva 0·019 by 0·021 mm.

Fig. 2. *M. expansa*, onchosphere from body cavity, mite dissected 14 May; larva 0·031 by 0·025 mm.

Fig. 3. *M. expansa*, mite dissected 27 May; the larva on the right, injured in dissection, measured 0·093 by 0·083 mm.

Fig. 4. *M. expansa*, the larger of the larvae in Fig. 3, at greater magnification, to show the germinal cells.

Fig. 5. *M. expansa*, mite dissected 10 July, larva 0·114 by 0·11 mm.

Fig. 6. *M. expansa*, mite dissected 7 July, larva 0·288 mm. long.

Fig. 7. *M. expansa*, mite dissected 17 August, cysticercoid 0·17 mm. in diameter.

Fig. 8. *M. expansa*, mite dissected 17 August, cysticercoid 0·18 mm. in diameter.

Fig. 9. *M. expansa*, the larva shown in Fig. 8, at greater magnification.

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ON SOME LARVAL STAGES OF TWO SPECIES OF ICHNEUMONIDAE, *BASSUS TETRAGONUS* THUNB. AND *HOMOCIDUS FISSORIUS* GRAV., PARASITIZING *SPHAEROPHORIA FLAVICAUDA* ZETT. (DIPTERA, SYRPHIDAE)

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From the Molteno Institute of Biology and Parasitology, University of Cambridge

(With 11 Figures in the Text)

Introduction

During a study of syrphid larvae I met with several instances of their being parasitized by hymenopterous larvae. The species of syrphid larvae most commonly parasitized, in my material, was *Sphaerophoria flavicauda*. From the puparia of this species I obtained two species of ichneumonids, which Mr J. F. Perkins, of the Natural History Museum, London, very kindly identified for me as *Bassus tetragonus* Thunb. and *Homocidus fissorius* Grav. Out of seven puparia of *Sphaerophoria flavicauda*, one adult of *Homocidus fissorius* and six of *Bassus tetragonus* were obtained. This paper therefore deals mainly with the larvae of the latter species.

Bassus tetragonus Thunb.

The most important synonymy of this species as given by Kerrich (1936) is: "*Bassus tetragonus* Thunb. = *flavolineatus* Zett. ♂ = *nemoralis* Holmgr. ♀ = *nemoralis* (Holmgr.) Thomas. ♂♀ = *tricinctus* auctt. non Grav."

Morley, who believed that this species was parasitic on the larvae of Syrphidae received a female, together with a puparium of *Platychirus albimanus*, its host, from Mr H. J. Charbonnier, by whom it had been bred on 6 August 1909, at Shepton Mallet in Somersetshire. Beyond this, nothing has been known of its development. In this paper I have attempted to describe some of its early stages, using the terms, early, intermediate and prepupal for the three stages found before the formation of the pupa. The nomenclature used in this paper is that adopted by Thorpe (1930) and Salt (1931).

Homocidus fissorius Grav.

Morley records *Homocidus fissorius* Grav. = *Bassus fissorius* Gr. = *Bassus punctatus* Bridg. = *Homoporus fissorius* Thoms. as bred from a syrphus larva, probably *Syrphus ribesii*, found on currant.

METHOD

As the host is transparent, by mounting it in distilled water on a slide and covering it with a cover-slip, the parasite may be seen within the body of the host, if the latter is parasitized. Neither the host nor the larva is harmed by this treatment, even though repeated several times. It may also be seen through the puparium, specially when the latter is moistened with water. The anatomy of the larva was studied from specimens in cedarwood oil, after dehydration, and also by means of sections stained in haemalum. 90 % alcohol was found to be a very suitable fixative.

BIOLOGY

As a rule only one parasitic larva is found within a syrphid larva. In one case however a puparium of *Sphaerophoria scripta* was found to contain eight prepupal stages of the parasites (Fig. 10), much smaller in size but similar in other respects to that described from *S. flavicauda*.

It is not possible to detect any trace of the puncture made in the body of *S. flavicauda* at oviposition. This is also true for other species of infected syrphid larvae. Only the third stage larvae of the host were found to be infected. The method of feeding in all three stages of the parasite is by suction, and can be seen by observing movements of the mouth. On examining a parasitized larva, although the mandibles of the parasite are well developed, all the tissues of the former are found intact, except the fat-body. It is presumed therefore that the movements of the mandibles indirectly bring about changes in the fat-body, which make it available for food. The decrease in the fat-body, however, is so small that the main source of nourishment of the early and intermediate stage larvae is probably the blood of the host.

When the puparium is formed, the soft parts of the host are broken up into small particles among which the parasite lies in its prepupal stage. This stage is characterized by the very rudimentary mandibles, hence the disintegration of the soft parts of the host must be brought about by some other means. Probably, as suggested by Timberlake (1912) in the case of *Limnerium validum* (Cresson), the parasite brings about pathological changes in the tissues of the host in this stage.

The prepupal stage increases very rapidly in size, a bubble of gas appears in the liquefied tissues of the host, and by the time the pupa of the parasite is formed, no trace of host tissue remains. Infected puparia are slightly narrower than the non-infected ones. The adult ichneumonid escapes by making a hole, with a crenulated margin, on the dorsal surface of the broad anterior end of the puparium.

The moulted skins of the parasite are not always found in the body of the host, and only recent exuviae of the head capsule will be found. The most probable explanation of this phenomenon is that of Pantel (1910), who believed that exuviae are destroyed by phagocytosis.

MORPHOLOGY OF DIFFERENT STAGES OF *BASSUS TETRAGONUS*

Early stage larva (Figs. 1 and 3). Length about 1·4 mm.; maximum width 0·3 mm. (at the fourth body segment). The head and the thirteen body segments clearly defined; the head is more sclerotized than body, and yellow or pale brown in colour. It is about 0·285 mm. long, flattened dorso-ventrally

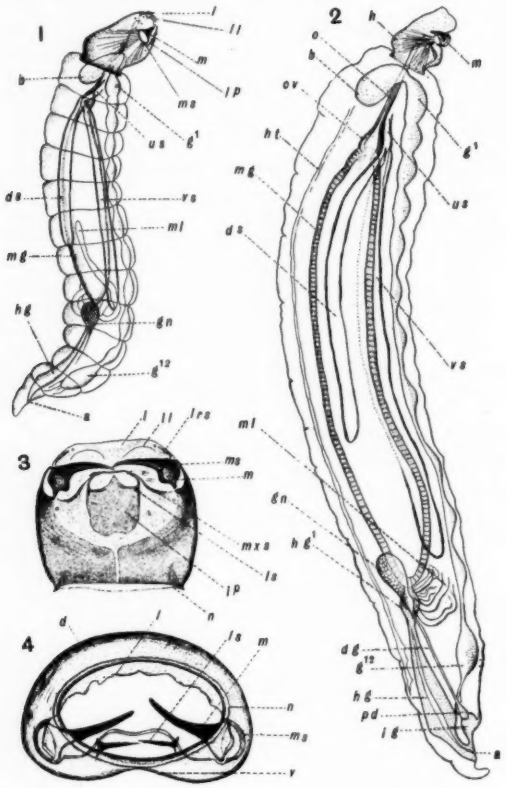


Fig. 1. Lateral view of early stage larva of *Bassus tetragonus*. ×44.
Fig. 2. Lateral view of intermediate stage larva of *B. tetragonus*. ×37.
Fig. 3. Ventral view of head-capsule of early stage larva of *B. tetragonus*. ×82.
Fig. 4. View through the occiput of moulted head-capsule of early stage larva of *B. tetragonus*. ×92.

and inclined ventrally. The facial "rods" are inconspicuous, except the semicircular mandibular strut (*ms*), the ends of which have minute concavities, into which the condyles of the mandibles (*m*) fit. This semicircular strut for the articulation for the mandibles is single, though in some other larvae as shown in Thorpe's figures, it is formed of two separate rods, i.e. mandibular

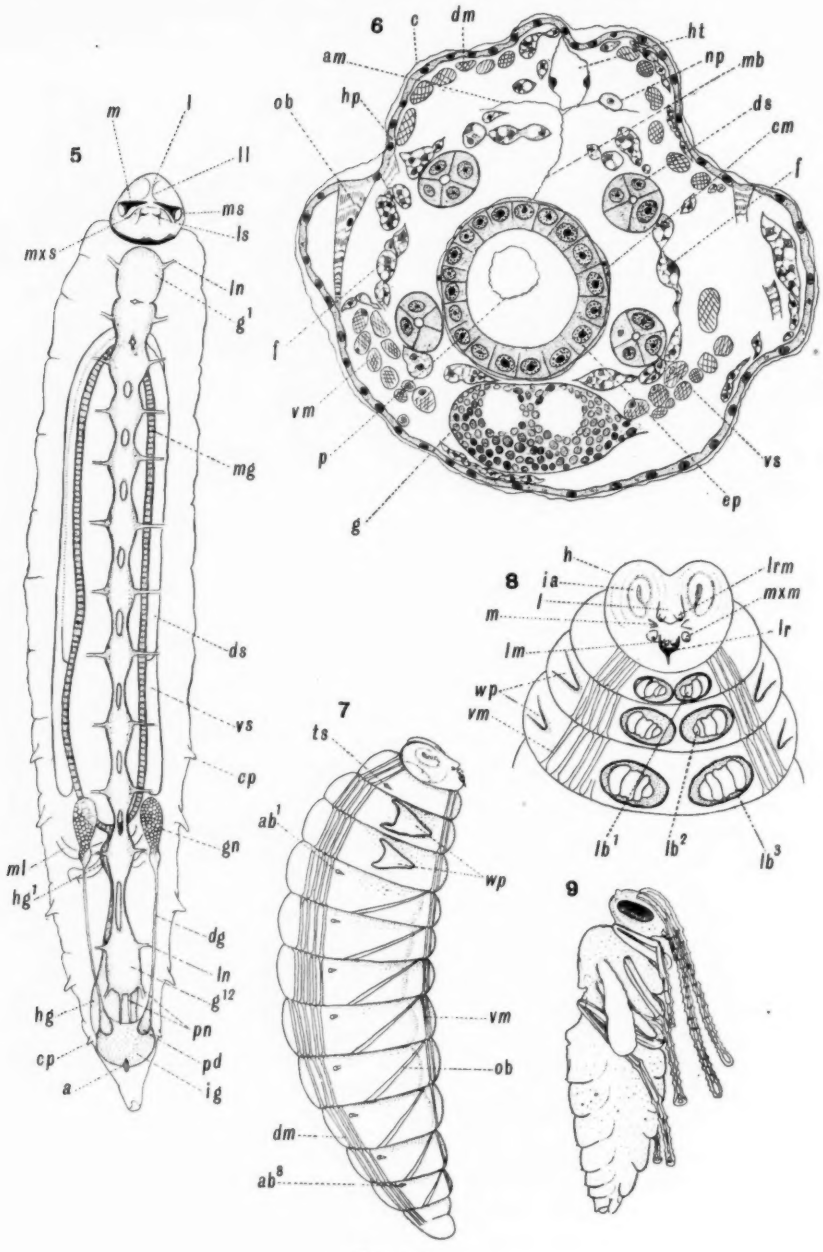
and labral struts. The mandibles are brown in colour, pointed, with slightly curved extremities and a broad base. The labrum (Figs. 1 and 3*l*) is situated under the anterior margin of the head capsule. It is thin and colourless and bears two swellings, the lateral lobes (Figs. 1 and 3, *ll*). The maxillary struts arise as pale and slender thickenings near the lower end of the mandibular strut and are directed towards the mouth-opening. The labial struts are also slender thickenings and have three tapering arms. The labral, maxillary and labial sensillae are not present at this stage. The region of the labial sensillae is occupied by a subquadrate space, the anterior side of which is prolonged forwards.

The integument of the body is colourless and transparent. The last body segment is conical, and is only slightly prolonged. When seen from the side the larva appears S-shaped. It moves freely in all parts of the body of the host.

Intermediate stage larva (Figs. 2 and 5). Length about 3·17 mm.; greatest thickness of the body 0·55 mm. The length of the head is the same as that of the first stage larva (0·285 mm.). In shape it differs only slightly from the early stage. The subquadrate labial area is not as pronounced as in the early stage. It moves about freely in the body of the host.

The change in the general appearance of the whole larva is clearly seen in the Figs. 2 and 5. The most important difference, however, is the occurrence in this stage of conical projections on the last five body segments, which are directed backwards and are not seen in the early stage. Similar appendages have been seen by other authors, but only in the first stage larvae of some ichneumonids.

Prepupal stage (Figs. 7 and 8). Length 5–6 mm., breadth 1·8–2 mm. Unlike the other stages the cuticle of the head is very thin and colourless, divided into two lateral lobes by a median anterior depression. The body, as in the first two stages, consists of thirteen segments. On the antero-ventral part of the head the unfolding of the imaginal disks of the antennae may be seen. A little behind this is the labrum with a pair of labral sensillae. At the sides of the mouth there are rudimentary colourless mandibles. Maxillary and labial sensillae are clearly seen. The labial ring is present as a brown Y-shaped strut, the anterior arms of which are level with the surface of the head. On the thin area between these arms lie the labial sensillae. The posterior sharply pointed and backwardly directed part of the labial strut projects from the surface of the head. The prepupa wriggles about, but its head always remains in the broad anterior part of the puparium of the host. At the sides of the second and third body segments, beneath the cuticle of the prepupa, are the developing triangular wing pads. On the ventral surface of the first three body segments oval depressions can be seen in the hypodermis. In each of these depressions thoracic leg buds can be seen. At this stage they appear to consist of four segments (Fig. 8, $lb^{1,2,3}$). In this stage nine pairs of functional spiracles can be seen: one on either side of the first thoracic segment, and the other eight pairs in the first eight abdominal segments. The imaginal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Figs. 5–9.

disks of the genitalia, consisting of two pairs, one above the other, are situated in the twelfth body segment, anterior to the anus.

The pupa (Fig. 9) is of the usual exarate type. It is 2 mm. in width at the thoracic region, and 5·2 mm. long.

ANATOMY

Alimentary canal. Two groups of muscles arise from the sides of the posterior part of the head and lead to the mouth and mandibles. The mouth leads into a narrow oesophagus, which ends in a small proventriculus situated at the level of the second body segment (Figs. 1 and 2). In the same segment the proventriculus leads into a wide thick-walled mid-gut. The mid-gut joins the hind-gut in the ninth segment, but at the point of junction the lumen is occluded. This condition exists in all stages till the formation of pupa. At its commencement the hind-gut is slightly swollen, and it is here that the malpighian tubes arise (Fig. 2, *hg*). The hind-gut is thick-walled and filled with fluid. It leads into the anus, which is situated in the thirteenth segment of the body. The cavity of the mid-gut of the first two stages contains liquid material, that of the prepupal stage a yellowish semi-solid material.

Salivary glands (Figs. 1, 2, 5 and 6, *ds*, *vs*). There are four tubular salivary glands around the mid-gut, equidistant from each other, the two dorsal ones shorter than the two ventral. The dorsal glands only reach the posterior margin of the sixth segment, while the ventral reach the ninth segment. A dorsal and a ventral gland of each side unite in the second segment, the two ducts thus formed again uniting at the base of the head to form the common salivary duct, which runs forwards to the mouth beneath the oesophagus. In transverse section the salivary glands are seen to consist of four thick glandular cells (Fig. 6, *vs*, *ds*). In the prepupal stage these glands are reduced in thickness as compared to the earlier stages.

Malpighian tubules (Figs. 1, 2 and 5, *ml*). These are four in number and their openings, as mentioned above, lie at the commencement of the hind-gut. They open in pairs, one pair on either side, into the hind-gut, the two openings of each side being close together. All four tubes run forwards to end in the sixth body segment. They are much narrower than the salivary glands in the larvae of early and intermediate stages, but in the prepupal stage the malpighian tubules are much thicker than the salivary glands, and reach the fourth body segment.

Legends to figs. 5–9.

Fig. 5. Ventral view of intermediate stage larva of *Bassus tetragonus*. ×44.

Fig. 6. Transverse section of intermediate stage larva of *B. tetragonus* through 4th body segment. (The irregular outline is due to the contractions of the body wall when the larva is dehydrated.) ×137.

Fig. 7. Lateral view of prepupal stage of *B. tetragonus*. ×14.

Fig. 8. Ventral view of head and three thoracic segments of above. ×20.

Fig. 9. Pupa of *B. tetragonus*. ×9.

Heart (Figs. 2 and 6, *ht*). This is a tubular structure running from the posterior end of the brain to about the eleventh body segment. In addition to the lateral wing muscles the heart is connected by means of a vertical membrane to the dorsal surface of the mid-gut (Fig. 6, *mb*).

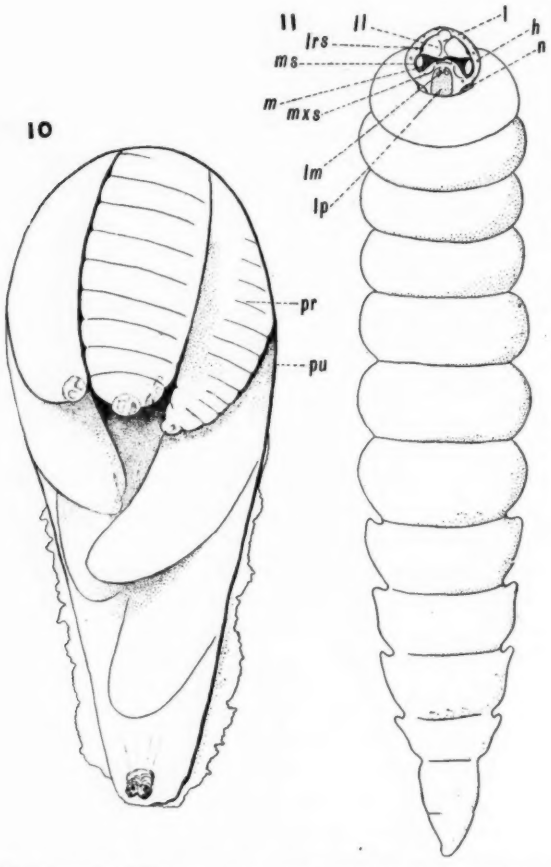

Fig. 10. Puparium of *Sphaerophoria scripta* containing eight parasites. ×14.
Fig. 11. Ventral view of a larva of *Homocidus fissorius*. ×44.

The respiratory system (Fig. 7) is present only in the prepupal stage. The number and position of the spiracles has already been mentioned. A tracheal trunk runs along each side of the body, from the first thoracic to the eighth abdominal segment. In addition to the branches leading to these from the spiracles there are others which ramify in the dorsal and ventral parts of each segment.

Fat-body. Of the three stages the prepupal stage is richest in fat-body. Portions of the fat-body of the intermediate stage larva are shown in transverse section in Fig. 6 (*f*).

Nervous system (Figs. 1, 2 and 5). The brain lies partly in the head and partly in the first segment in the early stage larva, in the first segment only in the intermediate stage larva, and in the head only in the prepupal stage larva. The ventral nerve chord consists of twelve ganglia, the first and last being larger than the others. The first ganglion is connected with the brain by means of thick commissures, but towards the posterior end the commissures between adjacent ganglia become longer and more slender. Each ganglion gives off two nerves, one from each side. In the last ganglion, however, in addition to these lateral ones, two pairs of nerves arise from the posterior border and travel towards the posterior end of the larva.

Gonads (Figs. 1, 2, 5, *gn*). As seen in the intermediate stage larva, these consist of a pair of solid club-shaped structures enclosed in a membrane. They are present on either side of the first (the swollen) part of the hind-gut. Each gonad is continued backwards by a fine duct, the expanded posterior end of which is embedded in the imaginal disk of the genitalia (Fig. 5, *ig*) within the ventral body wall.

The musculature is of the same simple type in all three stages, but is most clearly defined in the prepupal stage. There are four sets of longitudinal muscles, two dorsal and two ventral, which run from segment to segment (Figs. 7, 8). In addition to these is another series of muscles which run obliquely from the dorsal to the ventral side in the lateral part of each segment.

Homocidus fissorius Grav.

In addition to the larvae already described, one specimen of another species of ichneumonid (Fig. 11) was found parasitic on *Sphaerophoria flavicauda*. This was most probably *Homocidus fissorius*. Judging from the general shape and size, the stage of this larva corresponds to the intermediate stage of the larva of *Bassus tetragonus*. It differs from the larva of *B. tetragonus* in the following particulars: the head is more strongly chitinized; labral struts are present; the labial plate is a darker brown, longer and of a different shape anteriorly; a pair of labial sensillae is present; the sides of the eighth to eleventh segments are prolonged forwards (in the intermediate stage larva of *B. tetragonus* there are backwardly directed conical projections on the last five segments). This larva is 2·8 mm. long and at its thickest part (second body segment) is 0·51 mm.

Lettering of figures

a = anus
am = alary muscle of heart
b = brain
ab^1 = 1st abdominal spiracle
ab^8 = 8th abdominal spiracle
c = cuticle of body wall
cm = circular muscle of mid-gut
cp = conical projections from sides of abdominal segments
d = dorsal surface of head
dg = duct of gonad
dm = dorsal longitudinal muscles
ds = dorsal salivary gland
ep = epithelial cells of mid-gut
f = fat-body
g = ganglion of ventral nerve-cord
g^1 = suboesophageal or 1st ganglion of ventral nerve cord
g^{12} = 12th or last ganglion of ventral nerve cord
gn = gonad
h = head
hg = hind-gut
hg^1 = hind-gut (position of malpighian tubes)
hp = hypodermis of body wall
ht = heart
ia = imaginal antenna
ig = imaginal disk of the genitalia
l = labrum
lb^1, *lb*^{2}, *lb*^{3} = 1st, 2nd and 3rd imaginal leg buds
ll = lateral lobes of labrum
lm = labial sensillum
ln = lateral nerve
lp = labial plate
lr = labial ring
lrm = labral sensillum
lrs = labral strut
ls = labial strut
m = mandible
mb = vertical membrane connecting heart with mid-gut
mg = mid-gut
ml = malpighian tubules
ms = mandibular strut
mxm = maxillary sensillum
mxs = maxillary strut
n = occiput
np = pericardial nephrocyte
o = oesophagus
ob = oblique muscles
ov = oesophageal valve
p = peritrophic membrane
pd = posterior end of duct of gonad
pn = posterior nerves from 12th ganglion
pr = prepupal stages of the parasite
pu = puparium of host
ts = thoracic spiracle
us = union of dorsal and ventral salivary glands of one side
v = ventral surface of head
vm = ventral longitudinal muscle
vs = ventral salivary gland
wp = wing pads

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FURTHER NOTES ON *TRICHOGRAMMA SEMBLIDIS*

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(With 3 Figures in the Text)

An earlier paper (Salt, 1937) on the egg-parasite of the alder-fly, *Sialis lutaria*, reached three principal conclusions: (1) the insect is specifically distinct from *Trichogramma evanescens* Westwood and is to be called *T. semblidis* (Aurivillius); (2) its male exhibits a true dimorphism, since it occurs in two forms which are equally large and which differ constantly and fundamentally in several characters; (3) the appearance of the male sex is largely determined by the host—males reared on *S. lutaria* are of the apterous form, those from three species of Lepidoptera are of the winged form.

Since those conclusions were reached, work on the parasite has been continued for two years, chiefly to investigate the following five questions: (1) What further differences can be found to distinguish *T. semblidis* and *T. evanescens*? (2) Are there any intermediates between the two forms of the male? (3) Does the dimorphism persist if a pure strain is reared for many generations on one host? (4) Do any other hosts besides *Sialis* induce the apterous form? (5) By which kind of parthenogenesis does *T. semblidis* reproduce? These five questions are dealt with in the same order below.

1. Differences between *T. semblidis* and *T. evanescens*

No further morphological study of these two species has been made, and the characteristics to be considered here are all behaviouristic or physiological. The most interesting of them is the different behaviour of females of the two species in the presence of *Sialis* eggs. These eggs are laid in orderly masses of 500–700 eggs fastened side by side so that, with the exception of those around the edge of a mass, only the rounded end of each egg and its prominent micropyle is exposed (Fig. 1). Placed with such a mass, a lusty female of *T. semblidis* readily attacks eggs in any part of it, pushing her abdomen between the micropyles and her ovipositor through the end of the host. Females of *T. evanescens*, on the other hand, will not attack *Sialis* egg-masses, as is shown by the following experiments.

Ten females of *T. evanescens*, eager to lay and actively seeking hosts, were put with an egg-mass of *Sialis* in a small vial and kept under close observation under a microscope for 1 hr. The experiment was repeated on five different

occasions so that fifty females were tested. The parasites made a total of 189 crossings of the egg-masses, mounting them on one side, stumbling over the micropyles, and descending on the other side. In addition, the egg-masses were touched but not mounted ten times. In no case, however, did a parasite manifest any interest in the eggs—there was no examination of them as of prospective hosts and, of course, no attempt at oviposition.

To test the parasites, some of them were placed at the end of the hour with eggs of *Sitotroga* which they immediately parasitized. To test the egg-masses, they were exposed, when the females of *T. evanescens* had been removed, to females of *T. semblidis* and were at once accepted and attacked.

In another series of experiments, forty females of *T. evanescens* were put with five egg-masses of *Sialis* for 8 hr.; and in still another series, twenty-five

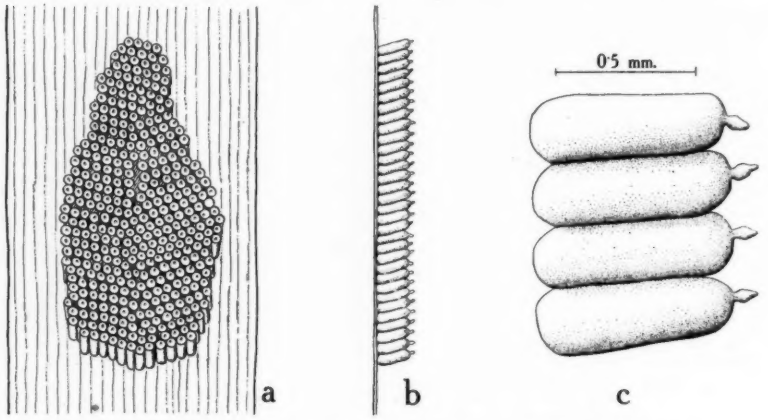

Fig. 1. Eggs of *Sialis lutaria*. *a*, an egg-mass; *b*, same in profile; *c*, four eggs enlarged to show their shape.

females were put with five egg-masses until they died. No examinations or attacks were observed, and no parasite progeny emerged. Since, however, *Sialis* eggs are almost always unsuitable for the development of *T. evanescens*, the last observation carries little weight.

From all these experiments and from others of a similar nature which need not be recorded in detail, it can be definitely concluded that females of *T. semblidis* and of *T. evanescens* differ in their behaviour with egg-masses of *Sialis*—*T. semblidis* readily attacks them, *T. evanescens* entirely neglects them.

The apparently obvious explanation of this result, that eggs of *Sialis* are attractive to *T. semblidis* but not to *T. evanescens*, is false. If individual eggs are separated from a mass and distributed singly on the substratum, the parasites react to them quite differently. In this case the *Sialis* eggs are accepted by *T. evanescens* but are usually rejected by *T. semblidis*.

In the course of several experiments, 707 separated eggs of *Sialis* have been attacked under observation and apparently actually parasitized by females of a pure strain of *T. evanescens*. This species will attack a *Sialis* egg even if it is attached to an egg-mass provided that most of its surface is free for examination. Thus, an egg fallen over from the side of an egg-mass, although still attached at its base, was parasitized; and on one occasion the apical egg of a mass which formed a very acute angle was examined by a female which twice momentarily assumed the attitude of oviposition but which eventually went off without ovipositing. It appears that *T. evanescens* must be able to examine most of the surface of its host before it will oviposit.

T. semblidis, on the contrary, does not readily attack separated eggs of *Sialis*, as is shown by the following experiments. From five to ten females of *semblidis* were put into a Petri dish with 100 separated eggs of *Sialis* and were kept under continuous observation. In seven experiments, twenty-six females were watched for ½ hr. and thirty-two females for 1 hr. Of these females, twenty-five had emerged from *Sialis* eggs collected in the open, and thirty-three were from a pure strain reared on eggs of *Sitotroga*. Only one of the 700 *Sialis* eggs was parasitized. Six others were momentarily attacked but were left before oviposition had occurred. Three more were examined. Yet these parasites were eager to lay, as demonstrated by their immediate acceptance of the eggs of *Sitotroga* and *Ephestia* with which they were put at the end of the experiment.

The two species, *T. semblidis* and *T. evanescens*, differ markedly, then, in their behaviour with eggs of *Sialis*. *T. evanescens*, which insists on examining the extent of its hosts before it will accept them (cf. Salt, 1935), rejects egg-masses of *Sialis* in which the size of the individual host is obscured, but accepts separated eggs which are exposed to its examination. *T. semblidis*, on the other hand, normally and naturally parasitizes egg-masses of *Sialis* but usually rejects individual *Sialis* eggs that have been artificially separated.

A physiological difference between the two species follows upon this difference in behaviour. *T. semblidis* finds eggs of *Sialis* suitable for its development and emerges in due course from *Sialis* eggs whether they are kept in a mass or, having been parasitized in a mass, are then separated. *T. evanescens*, on the contrary, is rarely able to develop in eggs of *Sialis*; and from 632 *Sialis* eggs parasitized by this species only twenty parasite progeny emerged, all of which were very feeble and died without being able to reproduce (Salt, 1938).

These twenty emergents included five males and fifteen females. The five males reveal still another difference between the two species. When a male *semblidis* emerges from a *Sialis* egg it is almost invariably of a distinct apterous form, quite different from the males that develop on lepidopterous eggs. The five males of *evanescens* that emerged from *Sialis*, however, were all of the ordinary form with fully developed wings and a consolidated flagellum bearing long setae. *Sialis* as a host, then, elicits a distinct apterous form of

semblidis, but has no effect on the appearance of the few *T. evanescens* that succeed in emerging from it.

In brief, in answer to our first question, the following behaviouristic and physiological differences between the two species can be adduced:

T. semblidis	*T. evanescens*
1. Attacks egg-masses of *Sialis*	Does not attack egg-masses of *Sialis*
2. Rarely attacks separated eggs of *Sialis*	Commonly attacks separated eggs of *Sialis*
3. Finds *Sialis* a suitable host	Finds *Sialis* an unsuitable host
4. Males emerge from *Sialis* in a distinct apterous form	The few males that emerge from *Sialis* are of the ordinary winged form

2. Intermediates between the two forms of the male

In the course of three years' work some hundreds of thousands of individuals of *T. semblidis* have been reared. It has not been possible to examine carefully more than a few thousand of these; but the emergents in certain generations and from particular experiments have been closely scrutinized and yield the following data.

A total of 3646 males of *T. semblidis* have been carefully examined. Of these, 1747 were perfectly normal apterous males, 1870 were entirely normal winged males, and only twenty-nine were in any way abnormal.

One of these last, which emerged from an egg of *Ephestia*, is a nearly normal apterous male of full size with the antennae and tarsi typical of the apterous male. The wing-lobes, however, are larger (Fig. 2*d*) than in a normal apterous male (Fig. 2*e*).

All the other twenty-eight specimens are undersized individuals which can be dealt with in three groups. The first group includes eight individuals of slightly less than normal size. Their wings are developed but not expanded (as in Fig. 2*a* and *b*) and their antennae (Fig. 3*b*) are clearly of the type of the winged male although there are fewer long setae and there is a tendency, not complete, to separate off two segments from the club.

The second group, of seventeen specimens, are small individuals having the wings reduced to lobes varying in degree of development between the two stages represented in *c* and *d* of Fig. 2. The two funicular segments of the antenna are clearly separated from the club, and the whole organ bears only a few long setae (Fig. 3*c*).

The third group is composed of three minute individuals which, measured in life, were only 0·26 mm. in length. No wings are visible on any of them, and the two funicle segments are completely separate from the club (Fig. 3*d*). The club itself is stouter than the club of an apterous male and resembles that of a female, while the few long setae that remain also make the antenna look much more like that of a normal female than of an apterous male.

The fact that these twenty-eight abnormal individuals are all less than full size immediately suggests that they are actually runts produced by star-

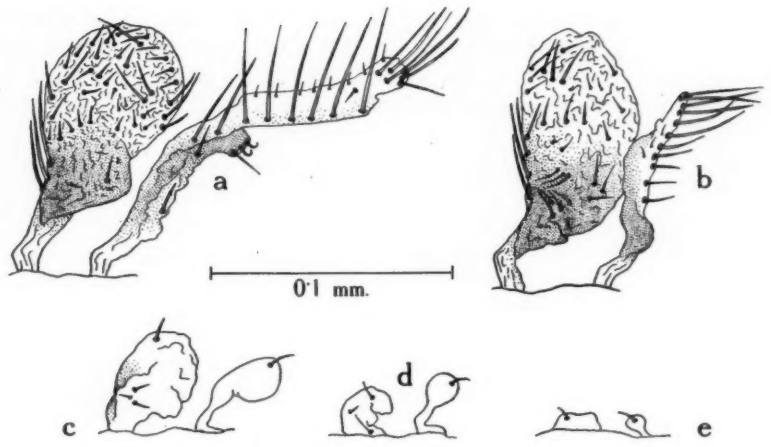

Fig. 2. Condition of the wings of *T. semblidis*. *a* and *b*, in runts of the first group; *c* and *d*, in runts of the second group; *e*, in a normal apterous male.

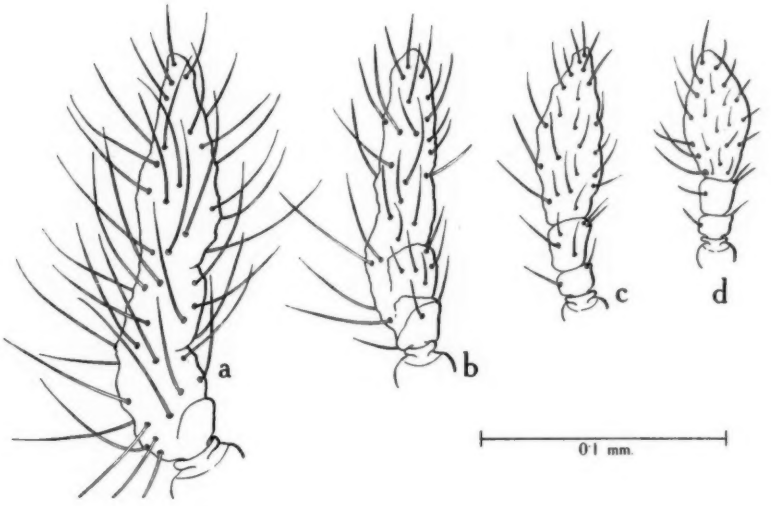

Fig. 3. Condition of the antennae of *T. semblidis*. *a*, in a normal winged male; *b*, *c*, and *d*, in runts of the first, second, and third groups, respectively.

vation such as are known to occur in *T. evanescens*. The characters in which they are abnormal (reduction of wings, loss of long setae from the antennae) indicate degeneration and support the same idea. The correlation of their size and their degree of degeneration, the smallest individuals showing the most extensive alteration in both wings and antennae, is further evidence of the operation of such an agent as starvation. Finally, in almost every case the particular cultures in which these individuals emerged show some other evidence besides their own appearance of superparasitism, either by the small size of other but normal individuals, both male and female, or by the known emergence of two parasites from a single egg of *Sitotroga* or *Ephestia*.

It is quite clear, then, that these twenty-eight specimens are not to be considered as normal forms intermediate between the usual winged and apterous males, but as degenerate individuals produced by starvation.

The occurrence of an abnormal apterous male, and the separation of the funicle segments of the runts, are interesting points that remain unexplained. The abnormality of the former, however, is so slight as to be insignificant, especially in the case of a dimorphism controlled by nutrition. The latter may be another case of arrested development if the compound club of the male *Trichogramma* is ontogenetically, as it certainly is phylogenetically, more recent than the divided form. In any case, the explanation of these two points is not essential for our present purpose.

This detailed account of twenty-nine abnormal specimens may tend to give a false impression of the stability of the two forms of the male. It must therefore be emphasized that of 3646 individuals examined only these twenty-nine, less than 1 %, were not perfectly normal specimens of one form or the other; and all but one of these twenty-nine are best looked upon as runts. The answer to the second question, then, is that there are no true intermediates between the two forms of the male *T. semblidis*, but intermediate types are simulated by degenerate individuals of small size produced by starvation.

3. The persistence of the dimorphism

A pure strain of *T. semblidis* was established in 1936 in the following manner. One female parasite that emerged from an egg-mass of *Sialis* collected in the open was put into a vial with eggs of *Ephestia kuehniella*. It had fifty-seven progeny, seven males and fifty females. One of these females was put with eggs of *Ephestia* and had thirty-five offspring, six males and twenty-nine females. Again one of these females was isolated, this time with eggs of *Sitotroga cerealella*, and had forty-six progeny, nine males and thirty-seven females. These forty-six individuals were placed in a stock dish with eggs of *Sitotroga cerealella*; and their descendants have been reared as a pure strain, exclusively on eggs of *Sitotroga* and continuously at 25° C., ever since (cf. Salt, 1937, p. 547).

As far as could be seen by observation of the stock dishes from time to time

the pure strain was composed exclusively of winged males and females. At the 17th generation a careful census was made. Twenty-five females were removed from the stock dish of the 16th generation and five of them were placed in each of five dishes of *Sitotroga* eggs. The twenty-five females had 588 progeny, 151 males and 437 females. The females were all normal and require no comment. Of the 151 males, 147 were entirely normal winged males; one was a normal apterous male; and three were runts.

At the 32nd generation a year had passed and the 1937 season of *Sialis* had begun so that it became possible to test the ability of the species to produce its apterous form on *Sialis* after thirty-two generations of the winged form on *Sitotroga*. In order to increase the number of critical (male) individuals, only virgin females were used. Parasitized *Sitotroga* eggs containing the 32nd generation were isolated in small vials and thirty-three virgin females were thus obtained. Twelve of these were put separately into dishes of *Sitotroga* eggs; twelve into dishes of *Ephestia* eggs; and nine into dishes containing egg-masses of *Sialis* laid in captivity. The first lot, on *Sitotroga*, had a total of 227 progeny, all males, of which 223 were of the normal winged form and four were runts. The twelve females put with eggs of *Ephestia* produced 246 progeny, all males, of which 236 were normal individuals of the winged form, three were normal apterous males, and seven were runts. The nine females with egg-masses of *Sialis* had 166 progeny, of which 165 were normal males of the apterous form and one was a slightly abnormal winged male.

The pure strain on *Sitotroga* continued to produce only winged males so far as was revealed by casual observation of the stock dishes from time to time, and, when the season of *Sialis* began in 1938, was in its 64th generation. Once again virgin females were obtained by isolating hosts before the parasites had emerged; and again some of these virgin females were allowed to parasitize eggs of *Sitotroga*, others eggs of *Ephestia*, and others egg-masses of *Sialis*. From twelve virgin females placed with eggs of *Sitotroga*, 192 progeny were obtained, all males; of which every one was a normal individual of the winged form. From six virgin females placed with eggs of *Ephestia*, there were ninety-three progeny, again all males; ninety-one of them normal winged males, and two of them runts. From nineteen virgin females placed with egg-masses of *Sialis* there were 175 progeny, all males and all normal, 174 of the apterous form and one of the winged form.

The main facts of this entire series of experiments are these: (1) A pure strain of *T. semblidis* reared on eggs of *Sitotroga cerealella* for over two years produced males almost exclusively of the winged form, as shown by casual observation of every generation and by a careful census in the 17th, 33rd, and 65th generations. The figures for male individuals reared from eggs of moths in these three generations are: 889 normal winged males, four normal apterous males, and sixteen runts.[1] (2) When placed with eggs of *Sialis*, however,

[1] These "runts" are included in the first and second groups of abnormal specimens described on p. 514.

members of this pure strain produced males almost exclusively of the apterous form, both in the 33rd and again in the 65th generation; the total numbers of males reared from *Sialis* in these two generations being 339 normal apterous males, one normal winged male, and one runt.

It must be concluded from these facts that the ability of *T. semblidis* to produce its apterous male form can remain latent through as many as sixty-four generations reared on *Sitotroga*, and yet find its full expression immediately when the parasite is reared on *Sialis*. The answer to our third question, then, is a decided affirmative.

It is to be noticed, further, that it is not only the dimorphism that remains latent but also the behaviouristic and physiological characteristics respectively of accepting *Sialis* egg-masses and of being able to survive on *Sialis* eggs. For 64 generations the ancestors of these female parasites had had experience exclusively of the separate eggs of *Sitotroga* sprinkled on paper. Yet, on being confronted with egg-masses of *Sialis*, they accepted and attacked them. For sixty-four generations this strain of *T. semblidis* had developed, as *T. evanescens* develops in the laboratory, in eggs of *Sitotroga*; yet it retained its ability, not shared by *T. evanescens*, to develop in eggs of *Sialis*.

4. Hosts of *T. semblidis*

Further evidence of the influence of the host on the form of the male has accumulated, of course, from all the experiments described above. Of the 3646 males that have now been carefully examined, thirty were reared on the special hosts listed below. All the others developed upon either *Sialis* or one of the three moths, *Sitotroga*, *Ephestia*, and *Barathra*. The distribution of the two male forms among these hosts is shown in Table I.

Table I. *The emergence of* 3616 *males of the two forms from eggs of moths and of* Sialis

Host	Normal apterous males	Normal winged males	Runts and abnormal males
Sitotroga cerealella	2	803	7
Ephestia kuehniella	11	986	17
Barathra brassicae	0	45	4
All three moths	13	1834	28
Sialis lutaria	1734	6	1

From eggs of *Sialis* there emerged 1734 apterous and six winged males; from eggs of the three species of moths were reared thirteen apterous and 1834 winged males. Among the 3587 normal individuals, then, only nineteen break the rule that the apterous form emerges from eggs of *Sialis* and the winged form from the eggs of moths. Of the twenty-nine abnormal individuals, eight certainly, and twenty-five very probably, are to be considered degenerate individuals of the winged form; one is certainly of the apterous form; and three are so small and degenerate as to defy classification. Among those that

can be assigned to one form or the other, only two are exceptional. In more than 99 % of the 3616 cases considered, then, the rule held that the apterous form emerges from eggs of *Sialis*, the winged form from eggs of *Sitotroga*, *Ephestia*, and *Barathra*.

The principal interest in considering the hosts of *T. semblidis* now lies in a search for hosts other than *Sialis* that will induce the apterous form. Attempts have been made to provide the parasite with hosts of three types: (1) hosts belonging to the same Order (Neuroptera); (2) hosts of similar feeding habits (predaceous); (3) hosts occurring in the same habitat (Coe Fen, Cambridge). The following species have been exposed to the pure strain of *T. semblidis*. Most of them combine the first two characteristics, being both neuropterous and predaceous.

Neuroptera[1]

Hemerobius lutescens Fabricius. Fifteen eggs were exposed to virgin females of *T. semblidis* and, although the parasites paid very little attention to them, at least two were eventually attacked and penetrated. Seven nymphs emerged and the other eight eggs shrivelled leaving no trace of parasite progeny.

Kimminsia subnebulosa (Stephens). Eggs of this species were not readily accepted and only thirteen attacks were made on the sixty-seven eggs exposed. No parasites developed in any of them.

Wesmaelius concinnus (Stephens). Most females of *T. semblidis* showed no interest at all in eggs of this species and only with persistence and the use of four or five hundred parasites were twenty-eight attacks observed. Of these, sixteen were abandoned in less than a minute, before the egg was penetrated; from four the female withdrew almost immediately after piercing the chorion; and only eight led to apparent oviposition. No parasites developed in these eggs.

Chrysopa flavifrons Brauer. Eggs of this species were readily attacked and were suitable. Of the forty-four parasites reared, thirteen were males. All of these were normal individuals of the winged form.

Chrysopa albolineata Killington. The forty individuals reared on this species included seventeen males, all of which were quite normal and of the winged form.

Hymenoptera

Selandria sixi Vollenhoven. Eggs of this sawfly were common on Coe Fen in early June and appeared to be a possible host for *T. semblidis*. Several eggs were exposed to the parasite but were not attacked.

Diptera

Tabanidae. The eggs of *Tabanidae* are of special interest for the point under discussion because they occur in marshy places and their larvae are predaceous. Several attempts were made to obtain Tabanid eggs for exposure to the pure

[1] I am obliged to Mr F. J. Killington for determinations of the five species of Neuroptera, and to Mr R. B. Benson for that of the sawfly.

strain of *T. semblidis*, but without success. Fortunately, however, Dr Ch. Ferrière has a series of *Trichogramma* bred from these hosts and kindly allows the following quotation from his letter: "Among my slides of *Trichogramma*, I have a series bred by myself from the eggs of a Tabanid found near a pool at Berne (Switzerland), of which all the males are winged and have the antennae of *T. semblidis* (with short setae, not longer than the scape)."

All of these hosts that are suitable, then, give rise to winged males of *T. semblidis*; and *Sialis lutaria* remains the only known host that induces the apterous form.

5. The reproduction of *T. semblidis*

According to Marchal (1936, pp. 510–14), the parasites he obtained from *Sialis* at Vincennes, Seine, included not only a bisexual strain, with production of males by unfertilized females, but also a strain capable of thelytokous parthenogenesis, of which unfertilized females were able to produce female progeny. That assertion is so completely at variance with observations of the *T. semblidis* occurring at Cambridge that an explanation of the discrepancy is desirable.

The parasite occurring in *Sialis* eggs at Cambridge is capable of arrhenotokous but not, so far as is known, of thelytokous parthenogenesis. Two kinds of evidence support this statement: (1) the composition of the families of fecundated females, and (2) the sex of the progeny of virgin females.

To provide the first kind of evidence, females from as many different sources as possible were isolated with hosts in order to detect any that gave rise to exclusively female families. A total of eighty-six females were so tested, of which seventeen came from the pure strain described above; twenty came originally from *Sialis* eggs collected in the field but with one or more intervening generations in the laboratory; and forty-nine came directly from seventeen different egg-masses of *Sialis* collected in the field. This material, therefore, represented many different natural "lines". Of the eighty-six families reared from these parasites, eighty-two contained males and four were exclusively female. The four exclusively female families cannot be considered significant, however, because they were all very incomplete. They consisted of only four, six, seven, and seven individuals respectively, while the average size of all the eighty-six families was 34·3. Three females from one of these four families were put with eggs of *Ephestia* but, unfortunately, they died without leaving any progeny. This evidence, then, is incomplete but, so far as it goes, belies the occurrence of thelytoky in *T. semblidis* at Cambridge.

The second kind of evidence is more conclusive. In the course of three years' work, families have been reared from seventy-nine virgin females of *T. semblidis*. The progeny numbered 1186 individuals, every one of which was male. In no case has a female offspring been reared from a virgin parent of *T. semblidis*.

This considerable body of evidence demonstrates clearly that the Cambridge *T. semblidis* reproduces by arrhenotokous parthenogenesis and, although as negative evidence it cannot be conclusive, it goes a long way to show that thelytoky does not occur.

There is, then, an actual discrepancy between the assertion of Marchal and the results of these experiments; and the question arises whether there are, within the species *T. semblidis*, both arrhenotokous and thelytokous strains, or whether the present observations or those of Marchal are at fault.

Failure to find thelytoky in *T. semblidis* at Cambridge, of course, does not prove that it does not occur at Vincennes, and it would be simplest to conclude that the French parasite is capable of both forms of parthenogenesis while the English is apparently capable of only one. Such a compromise recommends itself in the case of a difference of result with an investigator of Marchal's standing. Nevertheless, in fairness to the problem, four facts must be pointed out. (1) A thelytokous species of *Trichogramma*, that has often been called *T. evanescens*, exists, but it is specifically distinct from *T. semblidis*. (2) Marchal failed to distinguish between *T. semblidis* and *T. evanescens*. (3) He admits (1936, p. 497) that parasites escaped from some of his jars covered with fine muslin; and if one measures the mesh of the muslin used on his rearing tubes (Fig. 6, p. 458), comparing it either with the scale or with the host eggs, one can readily understand that small specimens of *Trichogramma* might easily escape from such a tube, or enter it. (4) He gives no data for isolated females but only for a mass culture. It is unpleasant to impugn the technique of so distinguished a scientist as Marchal, but it must be admitted that these facts lay the work open to the suggestion of contamination.

In answer to the fifth and final question, then, *T. semblidis* is certainly capable of arrhenotokous parthenogenesis, and has been said to include thelytokous strains; but the latter have not appeared in a large body of material from Cambridge and the work on which their existence was claimed requires corroboration.

SUMMARY

1. Several behaviouristic and physiological differences distinguish the two species *Trichogramma semblidis* and *T. evanescens* (tabulated on p. 514).

2. There are no true intermediates between the two forms of the male *T. semblidis*, but intermediate types are simulated by degenerate individuals of small size produced by starvation.

3. Among 1740 males reared from eggs of *Sialis* and 1847 reared from eggs of moths, there were only nineteen exceptions to the rule that the apterous form emerges from the former and the winged form from the latter.

4. The ability of *T. semblidis* to produce its apterous male can remain latent through as many as sixty-four generations reared on *Sitotroga* and yet find its full expression immediately when the parasite is reared on *Sialis*.

5. Several other species of Neuroptera have been parasitized, but *Sialis lutaria* remains the only known host that regularly elicits the apterous form.

6. *T. semblidis* is capable of arrhenotokous parthenogenesis. The statement that the species includes thelytokous strains requires corroboration.

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A STUDY OF THE OLFACTORY RESPONSES OF INSECT PARASITES TO THE FOOD PLANT OF THEIR HOST

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Picard and Rabaud (1914) appear to have been the first to draw attention to the fact that many parasitic Hymenoptera will attack the larvae of a variety of species belonging to different families and even to different orders of insects, provided that they all feed on the same species of food plant. That the host insect is not the only element in the environment of a parasitic insect is obvious; and from this it is equally obvious that general adult parasites must restrict their host-finding efforts to certain types of environment only. But it is only recently that there has been any definite proof of the mechanism of this restriction.

The present writer (Thorpe & Jones, 1937) showed, by means of olfactometer experiments, that the egg-laying females of the ichneumonid parasite, *Nemeritis canescens* Grav., are first attracted to the odour of the food of the host larvae, i.e. fresh oatmeal. Shortly afterwards Laing (1937) (whose paper gives a summary of the literature) published an account of the host-finding of two other hymenopterous parasites, the braconid *Alysia manducator* and the chalcid *Mormoniella vitripennis* which parasitize the immature stages of carrion-breeding Diptera.

In these insects odour is again a controlling factor, and it is clear from Laing's experiments that the parasites are attracted to meat, even though it does not contain, and never has contained, dipterous larvae and pupae.

But the sensory adaptations of parasites are not always as simple as such observations might suggest. The life histories of many insect parasites and predators are of great complexity, often involving nice adjustment to the time-table of the host insect. Then there is the necessity of finding food or quarters for hibernation or of locating an alternative host for the completion of the life cycle. In such instances a rigid response, olfactory or otherwise, to a single environmental factor or type of environment might be a fatal hindrance to successful adaptation. Many instances might be given, but it is not necessary to burden this brief account with examples from the literature.

A case in which the olfactory response to the food plant of the host is of particular interest is that of the parasites of the pine shoot moth, *Rhyacionia* (*Evetria*) *buoliana* Schiff. (Eucosmidae), an account of which was given by the senior author in 1930. The life history of this moth is briefly as follows. The adults first appear on the wing about the end of June, and eggs are laid on the

pine shoots at the end of July or early in August. They hatch in about a fortnight, and after a short period of wandering, the larvae commence feeding in a bud whorl. At this period they tend to withdraw themselves into cracks and crevices where they spin a thin protecting web over themselves, after which they proceed to excavate a feeding hole in the soft tissue underneath. It is probably during this period under the first protective web and during the early stages of feeding in the bud that most of the actual parasitization occurs. The first moult takes place at the age of three weeks, and in the second, or more usually third, instar the larvae block up the entrance of their burrow with a thick web and commence hibernation.

The larvae resume activity in March or April. Feeding is completed in the latter half of June or early in July, at which time the insects pupate among dead shoots or in a crevice under the thick silken web. The average duration of the pupal period is about 16 days. Since many of the parasites of the pine shoot moth emerge from their cocoons in June and July, long before there are young *buoliana* larvae available for oviposition, it follows that either the parasites must seek an alternative host on some other food plant, as for instance do *Pimpla examinator* F. and the three species of *Omorgus*, or else they must leave the pine trees for some considerable period in order to feed on the nectar of flowering plants (*Angelica sylvestris*, *Heracleum*, etc.) as is apparently the case with *Eulimneria rufifemur* Thoms. and *Pimpla ruficollis* Grav.

It was felt, therefore, that a study of the olfactory responses of these insects to pinene and pine oils, as well as to pine shoots themselves, might yield results of considerable interest. Accordingly in May 1938, through the kind co-operation of the officials of the Forestry Commission, a large supply, amounting to several hundredweight, of infested shoots of *Pinus sylvestris* was obtained from the forests at Swaffham, Norfolk. These shoots were picked over and the *buoliana* larvae and the parasite cocoons removed from them and placed in "emergence" cages. The parasites as they appeared were then transferred to cages where they were fed with honey and raisins and were supplied with a moist pad.

The experimental work was carried on by means of a modified McIndoo olfactometer, described in the previous paper, details of which need not be recounted here. All the usual precautions were observed and the observations were all made at 25° C. and 75 % relative humidity. As in previous experiments, in order to avoid any possible error due to the incidence of any slight bias in the apparatus, the position of the oil or other substance to be tested was alternated from one arm to the other at frequent intervals during each set of experiments. Thus in one half of each series the main stimulus would be on the right limb and in the other half on the left. In this way any errors due to imperfections of the apparatus cancel out.

Of the large variety of parasites which emerged, only two species, the Ophionine *Eulimneria rufifemur* Thoms., and the Pimpline *Pimpla ruficollis* Grav. were present in really large numbers. *Eulimneria rufifemur* was emerging

Table I. *Figures for responses of thirty-five females of* Pimpla ruficollis, *not more than* 1 *week old, to the odour of* Pinus sylvestris *oil in olfactometer*

Exp. no.	Date	Oil *P. sylvestris* 1/50	Blank	Total
1	6. vii. 38	11	14	25
2	,,	6	15	21
3	,,	6	9	15
4	,,	4	8	12
Total for insects 4 days old		27 = 37 %	46 = 63 %	73
5	8. vii. 38	7	14	21
6	9. vii. 38	7	14	21
7	,,	15	16	31
8	,,	5	5	10
9	,,	1	3	4
10	,,	6	8	14
Total for insects 6–7 days old		41 = 40·6 %	60 = 59·4 %	101
Total for all above experiments		68 = 39 %	106 = 61%	174

S.E. = ±3·79 %

from our material over a period of about three weeks, commencing on 17 June (possibly earlier), while *Pimpla ruficollis* was similarly appearing from the last week in June to mid-July. *Eulimneria rufifemur* lived about 3½ weeks in captivity, whereas some of the *Pimpla ruficollis* remained active for over 6 weeks. In the case of the former species, males and females are found in approximately equal numbers. In the latter the sex ratio is at present impossible to determine, since the males of three of the common pimpline parasites of *buoliana* are not as yet satisfactorily described. Although some males probably attributable to this species emerged, the females were greatly in excess, and reproduction is primarily parthenogenetic.

Preliminary experiments with both pinene and oil of *Pinus sylvestris*[1] showed that a dilution of one part in fifty with pure paraffin was the concentration which gave the most homogeneous results. Dilutions weaker than this produced no effect, while stronger concentrations were avoided for fear that they might have an irritant effect. For the purpose of olfactory experiments two drops of the mixture were absorbed on 2 sq. cm. of filter paper which was then placed in contact with the inner wall of the "bait tube" on one side of the instrument, the other side being left blank.

The accompanying tables show the results of these experiments and do not need a great deal of comment. Table I summarizes the responses of females of *P. ruficollis*, not more than 1 week old, and it will be seen that there emerges the somewhat surprising conclusion that the odour of oil of *P. sylvestris* is repellent to the insects. As will be seen from the standard error given, the result is quite significant.

[1] Details of the composition of *Pinus sylvestris* oil will be found in *Die Pflanzenstoffe* by C. Wehmer, vol. 2, pp. 1301–2, Fischer, Jena 1931. It contains 81 % of the Terpene, *d*–α Pinene, a characteristic constituent of the essential oils of conifers.

Table II. *Figures for responses of seventy females of* Pimpla ruficollis, *aged 32 days, to the odour of* Pinus sylvestris *oil in olfactometer*

	Exp. no.	Date	Oil *P. sylvestris* 1/50	Blank	Total
Series I	1	8. viii. 38	12	8	20
25 insects	2	,,	11	7	18
	3	,,	9	9	18
	4	,,	9	10	19
Series II	5	,,	11	5	16
25 insects	6	,,	8	14	22
Series III	7	,,	12	6	18
20 insects	8	,,	7	8	15
	9	,,	7	8	15
	Total		86 = 53·5 %	75 = 46·5 %	161

S.E. = ±3·94 %

Turning to Table II, we have results from insects aged 32 days, and from them it will be seen that the repellent effect is no longer manifest, in fact now the odour of the pine oil appears slightly attractive, although the figures are not significantly different from a random distribution. An almost identical result (56 % attraction to the oil) was obtained with another batch of insects aged 27–30 days, but owing to the small total number of results (87) and to the correspondingly high standard error the figures have not been set forth in tabular form.

Table III shows the results of similar experiments with insects aged six weeks, and it will be seen that now the attraction to the odour of *P. sylvestris* is more definite and can be regarded as significant in that it exceeds 50 % by more than twice the standard error.

Table III. *Figures for responses of forty-five females of* Pimpla ruficollis, *aged 6 weeks, to the odour of* Pinus sylvestris *oil in olfactometer*

Exp. no.	Date	Oil *P. sylvestris* 1/50	Blank	Total
1	23. viii. 38	17	14	31
2	24. viii. 38	12	10	22
3	,,	14	11	25
4	,,	13	9	22
5	,,	17	7	24
6	25. viii. 38	11	9	20
7	,,	13	8	21
8	,,	11	10	21
9	,,	9	5	14
Total		117 = 58·5 %	83 = 41·5 %	200

S.E. = ±3·54 %

Table IV gives results for insects of the same age when pinene is substituted for the oil of *P. sylvestris*.

It appears certain from these tables that during the life of the female *Pimpla ruficollis* a marked change takes place in the response to the odour of

oil of *Pinus sylvestris*. Comparing the results of Tables I and II by means of the formula

$$\chi^2 = \frac{(ad-bc)^2 N}{(a+b)(c+d)(a+c)(b+d)},$$

the value of χ^2 is found to be 6·919. Since in this case there is only one "degree of freedom", the value for χ^2 must be read from the column $n=1$ in Fisher's Table. In this column the value 3·841 corresponds with the value of $p=0{\cdot}05$. Thus a value of χ^2 greater than 3·841 implies that there is less than one chance in twenty that the two classifications are independent. For one "degree of freedom" a χ^2 of 6·919 corresponds to a P of less than 0·01, i.e. the probability that the differences are "due to chance" is less than one in one hundred. One is consequently justified in assuming that there has been a real change with age in the response to the odour of pine oil. It follows that if we compare the figures given in Table I with those of Table III, the differences have an even greater level of significance.

It can be shown that this change in the olfactory responses of the female is correlated with the degree of development of the ovaries. During the first week of life they are very minute, but towards the end of the period they are well developed, and the insect is ready to lay her eggs. It is impossible to say how far this relationship is one of cause and effect, but it is significant that the change in response agrees satisfactorily with what is known of the life history of the parasite, and it is correlated with that of its host.

The period when the insect is repelled by the odour of pine is that during which the host caterpillars are not yet available and during which the parasite probably leaves the pine trees to feed on the flowers of Umbelliferae and other plants. After about the third or fourth week of life, the host is in a stage suitable for attack, and the parasite presumably returns to the infested pine shoots.

Table IV. *Figures for responses of forty females of* Pimpla ruficollis, *aged 6 weeks, to odour of Pinene in olfactometer*

Exp. no.	Date	Pinene 1/50	Blank	Total
1	20. viii. 38	17	18	35
2	,,	16	10	26
3	,,	16	13	29
4	,,	23	13	36
5	21. viii. 38	17	16	33
6	,,	18	10	28
7	,,	5	20	25
Total		112 = 53 %	100 = 47 %	212

S.E. = ±3·43 %

Owing to the lower percentage of parasitism by *Eulimneria rufifemur* and to the greater difficulty experienced in keeping them alive in captivity, the number of experiments which it was possible to perform was not sufficient to allow of conclusive results being obtained. There is some indication, however, that

conclusions reached as a result of the experiments with *Pimpla ruficollis* also apply to *Eulimneria rufifemur*. The latter when newly emerged is repelled by the odour of pine extracts to almost exactly the same degree (62 %) as *Pimpla ruficollis*. Later (at the age of 3 weeks) an attraction of 60 % is shown to the odour of clean pine shoots in the olfactometer. Although the numbers are small, the difference between the two figures is greater than the value of S.E. × 2 by a clear margin, and the difference can, therefore, be regarded as significant.

Geraniol is an alcohol derived from Terpenes and known to be a constituent of a great variety of essential oils including those from certain conifers (Gildemeister & Hoffmann, 1928). Since this substance is also a well-known attractant for many insects a short series of experiments was performed with *Pimpla ruficollis* of ages 8–10, 23 and 33 days, using very dilute Geraniol as the test substance. In every case, however, there was a definite repulsion (58–63%) exerted by the odour of the Geraniol.

Summary

1. *Pimpla ruficollis* is a parasite of the pine shoot moth *Rhyacionia* (*Evetria*) *buoliana* Schiff. (Eucosmidae) which emerges from its host a considerable time before the next generation of larvae are ready for oviposition. It seems that during this period the parasites leave the pine trees and feed on the flowers of certain Umbelliferae and probably other plants. Only after 3 or 4 weeks do they return to the pine trees where the shoots now contain larvae ready for egg laying.

2. It has been shown by means of olfactometer experiments that during this first period of the adult life, the parasites are repelled by the odour of oil of *Pinus sylvestris*. At this time the ovaries are very small and are probably not ready for the production of eggs. After the third or fourth week of life the females become attracted by the oil of *P. sylvestris* and the ovaries are now relatively large.

3. Since Geraniol is known to be a constituent of certain conifers, this substance was also tested. It was, however, found to be invariably repellent to the parasites.

4. There is some evidence that conclusions reached as a result of experiments with *P. ruficollis* also apply to the ophionine ichneumonid *Eulimneria rufifemur*.

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MORPHOLOGICAL AND TAXONOMIC STUDIES ON MAMMALIAN TRYPANOSOMES

VI. FURTHER OBSERVATIONS ON THE ABSENCE OF THE KINETOPLAST IN *TRYPANOSOMA EVANSI*

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(With 1 Figure in the Text)

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I. A new akinetoplastic strain

In a previous paper (Hoare & Bennett, 1937) an account was given of the spontaneous occurrence, in the Anglo-Egyptian Sudan, of four strains of the camel trypanosome, *Trypanosoma evansi*, entirely devoid of the kinetoplast (=kinetonucleus).[1] Unfortunately, in none of these cases was it possible to keep the trypanosomes under further observation, as the camels in question were either lost sight of or had undergone treatment and, therefore, nothing could be ascertained regarding the duration of the "akinetoplastic"[2] condition in such strains, nor was it known whether they behaved differently from normal ones in the mammalian hosts. Since then some new investigations have been made and we are in a position to throw more light on these problems.

In April 1937, a new (5th) akinetoplastic strain was encountered in a camel from Gedaref. This camel was one of a batch of twelve heavily infected ones from the same locality, but the other eleven camels showed *normal* trypano-

[1] The terminology of this structure is discussed in the preceding paper of the present series (Hoare, 1938).

[2] In conformity with the substitution of the term *kinetoplast* for kinetonucleus we now adopt the term *akinetoplastic* to denote the absence of this structure, the new term being synonymous with "akinetonucleate" and "ablepharoplastic".

somes. The camel in question was kept under observation for about four months, in the course of which trypanosomes appeared and disappeared from its blood at irregular intervals, while the camel itself was gradually losing condition. During the whole of this period the flagellates remained akinetoplastic.

This strain was inoculated at various times from the original camel to (1) another camel, (2) a horse, (3) two donkeys, (4) two bulls and (5) gerbils.

In the passage camel all the trypanosomes were also devoid of the kinetoplast during the fortnight that it was under observation before being treated and cured.

In the horse only a few trypanosomes could be detected in the course of about three months after inoculation, and their structure was not ascertained, but in gerbils infected from time to time from the horse the trypanosomes were invariably akinetoplastic.

As regards the donkeys and the bovines, these never revealed a visible infection in the blood, but gerbils inoculated from these animals developed infections with akinetoplastic trypanosomes.

In addition to the ungulates referred to above the akinetoplastic strain was maintained in gerbils, starting from the original camel. In Khartoum it was kept under observation till December 1937, i.e. for eight months, during which eighteen passages through these rodents were made. Gerbils of the 5th passage were sent by air to London where the strain has been kept (from June 1937) by passages through mice. At the time of writing (September 1938) the strain is in the 50th passage through mice (= 55th passage since it was isolated from the camel), having been maintained for one year and a half in laboratory animals. It was established, by regular examinations of the blood of these animals in stained films, that throughout the whole of this period the strain has retained the akinetoplastic condition unaltered.

Clinical observations on the animals infected with the akinetoplastic strain —both under natural and experimental conditions—have shown that it behaves in them in exactly the same manner as the normal cameline strains of *T. evansi*: in camels it produces a wasting disease, with progressive loss of condition; its effect upon bovines is slight, while it does not affect equines at all; in gerbils the infection usually lasts about ten days and terminates fatally.

From the foregoing account it would appear that in the present strain of cameline *T. evansi* the absence of the kinetoplast has been established as a permanent feature, unaffected either by fluctuation in the number of trypanosomes occurring in the course of the infection in the natural host, or by their passage through series of similar and dissimilar hosts (camel-camel; camel-donkey-gerbil; camel-horse-gerbil; camel-bull-gerbil; camel-gerbil-mouse). As regards the virulence of this strain, it does not differ from that observed in normal strains.

From these and from previous observations on similar strains (Hoare & Bennett, 1937) there can be no doubt that akinetoplastic strains of *T. evansi* arise spontaneously in Sudanese camels under natural conditions, and may

continue to "breed true" for what seems to be an indefinite period, as in the case of the South American "species", *T. equinum*.

It may be added here that the akinetoplastic equine strain of *T. evansi* (= *T. marocanum*), originally described by Wenyon (1928) and later by us (1937), is still being maintained at the Wellcome Bureau of Scientific Research. More than ten years have now elapsed since it became akinetoplastic, but the strain still retains this characteristic unchanged.

Though we have made no further advance toward the discovery of the cause of the loss of the kinetoplast in individual trypanosomes, some new light has been thrown on the mechanism which governs the fluctuation of the number of trypanosomes devoid of this organ and which may be ultimately responsible for the production of akinetoplastic strains. The observations on which these conclusions are based will be considered in the section to follow.

II. FLUCTUATION IN THE PROPORTION OF AKINETOPLASTIC TRYPANOSOMES

(1) *Preliminary remarks*

It is well known that akinetoplastic individuals may be present in various proportions in normal strains of mammalian trypanosomes. This question has been fully dealt with in an earlier paper (Hoare & Bennett, 1937), in which numerous examples of their occurrence in different species are adduced. Among the *normal* cameline strains of *T. evansi* from the Sudan examined up to the present the percentage of akinetoplastic individuals was found to vary from 1 to 6 (*loc. cit.* Table I), which is within the range found for other strains of this species in the Old World.

After the isolation of the akinetoplastic strain (V) described in the preceding section it was decided to undertake a parallel comparative study of a normal or typical cameline strain of *T. evansi* from the same locality. A strain was accordingly isolated at the beginning of January, 1938, from a camel which acquired its infection in Kordofan. The blood of this camel contained few trypanosomes. In stained films, examined on one occasion, the strain appeared to be quite typical, in that it contained only 1 % akinetoplastic forms. However, in the first four passages of this strain through gerbils the percentage of akinetoplastic trypanosomes underwent considerable fluctuation, rising at one time to 71 and subsequently falling to 3 (see Table I). This extraordinary and unexpected fluctuation seemed to open new possibilities in the study of the nature of the akinetoplastic condition, and we accordingly modified our original programme of investigation.

In the first place, it was decided to examine the strain in question in successive passages, at more or less regular intervals of time, in order to determine the extent and duration of fluctuation in the proportion of the akinetoplastic forms. Secondly, it was proposed to consider the bearing of this phenomenon upon the production of totally akinetoplastic strains, and, thirdly, attempts

were to be made to elucidate, if possible, the causes and mechanism of the fluctuation.

Before proceeding with these observations it is necessary to make a few preliminary remarks regarding the present strain. One of its chief features is its extremely low virulence for laboratory animals. As stated above (p. 530), infection with the cameline strains of *T. evansi* usually runs an acute course in gerbils, lasting about 10 days, whereas the infection with the present strain was chronic in these animals, having persisted, in the 5th passage (see Table I), over 4 months, in the course of which the trypanosomes varied considerably in numbers at different times, sometimes disappearing altogether from the circulation and then reappearing at irregular intervals. However, the chronic type of infection in gerbils is not peculiar to this strain, for Fry (1911), working with the same trypanosome, noted that the course of infection in gerbils varied from an acute type lasting 10 days to a chronic form lasting 93 days. The variation in the duration of the infection was, therefore, comparable to that observed by us in the two strains described in the present paper. We have also experienced considerable difficulty in infecting mice with our strain. Repeated inoculations from gerbils—sent from Khartoum to London—were required before infection could be established in mice.[1]

In these animals the course of the infection was very erratic: in most cases it was quite transient, the trypanosomes appearing in the blood on one or two days and then disappearing entirely; in some cases, however, they were present in large numbers for considerable periods of time (cf. Table I: passages XI, XVIII, XVIII *a*, XXII). The maximum period during which trypanosomes could be found in the mice was 35 days, while in the totally akinetoplastic strain (V) of this trypanosome, described in the preceding section, the average duration of the infection in these animals was about 20 days, with a minimum of 7 and a maximum of 31 days. As regards the pathogenicity of these strains, the one under consideration killed some of the gerbils only after about 4 months and was rarely fatal to mice, whereas strain V invariably ended in the death of the experimental animals.

(2) *Extent of fluctuation*

We now turn to the actual observations on the fluctuation in the proportion of akinetoplastic forms in our strain. At the beginning, before the significance of this phenomenon was realized, the observations were conducted at irregular intervals, but later daily examinations were made in all suitable passage animals. In each case one hundred individuals were counted and the number of akinetoplastic forms among them was noted.

The results of all these observations are shown in Table I, while Fig. 1 gives a graphic representation of the fluctuation in four selected passages.

[1] We take this opportunity to express our gratitude to Mr C. E. Berry, Chief Laboratory Assistant, Wellcome Bureau of Scientific Research, whose care and perseverance in the maintenance of the strains has greatly facilitated our work.

Table I. *Fluctuation in the percentage of akinetoplastic forms in* Trypanosoma evansi

Table I. *Fluctuation in the percentage of akinetoplastic forms in* Trypanosoma evansi

In each observation the roman numeral on the left indicates the number of the passage; the figures in brackets refer to date of examination (day and month), which is in some cases preceded by the date of inoculation from last passage; the numeral on the right (in heavy type) denotes the percentage of akinetoplastic forms.

| | | |

A glance at the Table and the graphs gives an idea of the amplitude of fluctuation in the proportion of akinetoplastic forms, which varies from zero to a maximum of 71% in the whole range of observations. Another point that strikes the eye is the difference in the degree of fluctuation occurring in parallel passages. For instance, in one gerbil of passage V (cf. Table I and Fig. 1, *B*) there are four peaks, the percentage of akinetoplastic forms rising steeply (to 30, 40, 46, 43) and falling to a low level (9, 17, 3, 1) in successive waves: in this case the number of akinetoplastic trypanosomes remained high during the week preceding the death of the host. On the other hand, in a sister-gerbil of the same passage (V *b*), inoculated from the same gerbil as the preceding one (cf. Table I and Fig. 1, *A*), the percentage of akinetoplastic forms shows a

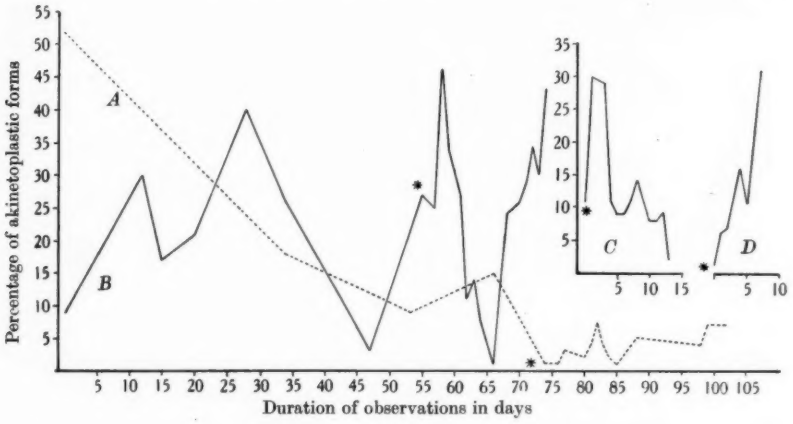

Fig. 1. Graphs showing fluctuation in the proportion of akinetoplastic trypanosomes. The numbers on the ordinates indicate percentage of akinetoplastic forms, those on the abscissae denote duration of observations in days. *A* (interrupted line) = passage V*b* (gerbil); *B* = passage V (gerbil); *C* = passage XVIII*a* (mouse); *D* = passage XVIII (mouse). Asterisks (*) indicate starting points of daily records.

gradual decline from 52 to a "normal" level (1–7 %), at which it remained till the death of the animal. This type of fluctuation was observed in several passages (cf. Table I: passages XI, XXI), while on other occasions there was a fairly regular rise in the percentage (cf. Table I, passage XVIII, and Fig. 1, *D*). In most cases, however, the fluctuation was as irregular as in gerbil V [e.g. passage XVIII *a* (cf. Table I and Fig. 1, *C*), passage XXII (cf. Table I)]. On several occasions the percentage remained at a constant high level throughout the period of observation (cf. Table I, passages XII and XX).

From these data it is seen that there is no uniformity or regularity in the fluctuation of the number of akinetoplastic forms in this strain, even in parallel series observed simultaneously and under identical conditions.

(3) *Bearing upon the origin of akinetoplastic strains*

It is well known that akinetoplastic strains of trypanosomes can be produced experimentally with the aid of certain chemicals which eliminate the kinetoplast either by destroying it directly or by incapacitating it for further reproduction. A discussion of the investigations on this subject and references to the literature will be found in our previous paper (Hoare & Bennett, 1937). As regards similar strains that had arisen spontaneously—both under laboratory and under natural conditions—the agency causing the disappearance of the kinetoplast could not be ascertained.

Though we have now had under continuous observation another "natural" akinetoplastic strain (V) and a strain with a wide fluctuation in the proportion of trypanosomes devoid of the kinetoplast, we must confess that we are still far from the solution of the nature of this phenomenon.

However, though the initial cause of the disappearance of the kinetoplast remains unknown, the present investigation has thrown further light on the factors that may possibly be instrumental in the subsequent production of strains of trypanosomes devoid of this organ. We have already (*loc. cit.* p. 46) adopted the view that the production of akinetoplastic trypanosomes is probably due mainly to an irregular form of division in the course of which the kinetoplast fails to divide, so that one of the daughter individuals lacks this organ. If it is assumed that the same disability affects the kinetoplast of the second daughter individual and its progeny, there will be a tendency for akinetoplastic individuals to be produced in progressively increasing numbers in successive generations (i.e. after successive divisions), the ratio of aberrant individuals to normal ones being as $(2^n - 1) : 1$, where n = number of divisions. This view involves a second assumption—justified by the continued conservation of totally akinetoplastic strains—viz. that an individual deprived of the kinetoplast tends to propagate similar individuals or "breed true".

In accordance with this hypothesis it was suggested that "it is conceivable, on theoretical grounds, that an akinetonucleate [= akinetoplastic] strain might arise from a normal one through the chance inoculation by the natural vector of a single trypanosome devoid of the kinetonucleus" (*loc. cit.* p. 54). Since normally the proportion of akinetoplastic forms in *T. evansi* is low (1·0–6·0 %) it is obvious that the chances of akinetoplastic strains arising from aberrant individuals in the manner suggested above are very remote. However, the discovery of the present strain, in which the proportion of akinetoplastic forms may rise above 70 %, lends considerable support to our contention, for in the case of such strains as ours there occur favourable periods when the chances of an akinetoplastic trypanosome being inoculated into camels by the natural vector are actually higher than the chances of introducing a normal individual (in our case the ratio of aberrant to normal trypanosomes was 2·5:1 on one occasion: cf. Table I, passage III *a*).

Furthermore, in view of the wide range of fluctuation observed in the

present strain, it is possible that its amplitude may eventually reach the upper limit (100 %) and thus involve all the trypanosomes present. In that case a totally akinetoplastic condition would result within the host directly, without the intervention of the vector. It is conceivable, therefore, that aberrant strains can be produced by both the methods suggested. The fluctuating strain is being kept under continuous observation in the hope that conditions favourable for the verification of this hypothesis will arise some day.

It should be noted that the foregoing conclusions are based on observations made in experimental animals, gerbils and mice, and it might be argued that they do not necessarily apply to the natural host, the camel, since there is no direct evidence of a wide fluctuation in the proportion of akinetoplastic forms in this animal, the single examination of the blood of the camel from which the present strain was isolated having revealed a "normal" picture. However, the fact that totally akinetoplastic strains have been found by us repeatedly in the camel itself, indicates that the aberrant condition arises and is retained in the natural host, and is not due to change of hosts. Moreover, this condition has never been previously recorded in gerbils infected with *T. evansi*, though these animals are commonly used for this purpose in the Sudan. We have also seen that an akinetoplastic strain isolated from a camel remained unaltered in successive passages through heterogeneous animals. If we add to these facts the occurrence of an aberrant strain of *T. evansi* which had arisen spontaneously in mice (*vide supra*, p. 531) it will be clear that there is no evidence that the type of host plays any part in the production and retention of the akinetoplastic condition in trypanosomes.

(4) *Mechanism of fluctuation*

We now turn to a consideration of the mechanism responsible for the fluctuation in the proportion of akinetoplastic trypanosomes in our strain. For the elucidation of this question we have made daily observations in a number of passages and have selected those in which the degree of fluctuation was the greatest. In the discussion to follow it will be convenient to use the symbol *K* to denote the presence of a kinetoplast, and *Ak* to denote its absence.

From Tables I and II (at *a*) and Fig. 1 it will be seen that in passage V the percentage of *Ak* forms varied in the course of 6 days as follows: 25–46–34–27–11, while in passage XVIII *a* the following variation occurred in the course of five days: 11–30–29–11. In accordance with the views expressed above (p. 535) it was thought that irregularities in the method of division might afford some clue to the wide fluctuations observed; therefore 100 dividing[1] individuals were examined on each of the days for which the percentage of non-dividing *K* and *Ak* forms had been estimated, and the percentage of various types of division seen was noted. Of these there were three: (1) in which the kinetoplast had given rise to two daughter kinetoplasts ($K+K$); (2) in which the kinetoplast

[1] Only those stages of division were taken into consideration in which two flagella were present but the body itself had not yet undergone fission.

Table II. *Relation of daily fluctuations in the proportion of akinetoplastic trypanosomes to variations in the type of division*

The letters between the two columns refer to the corresponding groups of both (indicated by dotted lines) and have the following meaning: (*a*) percentage of forms with (*K*) and without (*Ak*) kinetoplast in non-dividing trypanosomes; (*b*) percentage of forms with two (*K*+*K*), one (*K*+*Ak*) and no (*Ak*+*Ak*) kinetoplasts in dividing trypanosomes on same date as *a*; (*c*) estimated number of trypanosomes with (*K*) and without (*Ak*) kinetoplast after *n* divisions (value of *n* shown in brackets at *c*); (*d*) ratio of normal (*K*) to akinetoplastic (*Ak*) forms after *n* divisions.

Gerbil: passage V		Mouse: passage XVIII *a*
(5. v. 38)		(24. vi. 38)
K 75 — *Ak* 25	(*a*)	*K* 89 — *Ak* 11
K+*K* 55 — *K*+*Ak* 26 — *Ak*+*Ak* 19	(*b*)	*K*+*K* 59 — *K*+*Ak* 11 — *Ak*+*Ak* 30
K 14106 ($n=8$) *Ak* 11494	(*c*)	*K* 129 ($n=1$) *Ak* 71
54 — 44	(*d*)	70 — 38
(6. v. 38)		(25. vi. 38)
K 54 — *Ak* 46	(*a*)	*K* 70 — *Ak* 30
K+*K* 61 — *K*+*Ak* 17 — *Ak*+*Ak* 22	(*b*)	*K*+*K* 62 — *K*+*Ak* 2 — *Ak*+*Ak* 36
K 15633 ($n=8$) *Ak* 9967	(*c*)	*K* 126 ($n=1$) *Ak* 74
66 — 42	(*d*)	71 — 42
(7. v. 38)		(27. vi. 38)
K 66 — *Ak* 34	(*a*)	*K* 71 — *Ak* 29
K+*K* 73 — *K*+*Ak* 3 — *Ak*+*Ak* 24	(*b*)	*K*+*K* 71 — *K*+*Ak* 5 — *Ak*+*Ak* 24
K 18691 ($n=8$) *Ak* 6909	(*c*)	*K* 147 ($n=1$) *Ak* 53
73 — 27	(*d*)	89 — 32
(9. v. 38)		(28. vi. 38)
K 73 — *Ak* 27	(*a*)	*K* 89 — *Ak* 11
K+*K* 79 — *K*+*Ak* 0 — *Ak*+*Ak* 21	(*b*)	
(10. v. 38)		
K 89 — *Ak* 11	(*a*)	

had failed to divide, giving rise to the condition *K*+*Ak*; and (3) which was akinetoplastic (*Ak*+*Ak*). Amongst these three types of division it is obvious that the first two belong to typical (*K*) individuals, the second, however, being the irregular form of division; while the third type represents the division of akinetoplastic forms (*Ak*).

The results of the counts in the two passages are shown in Table II (at *b*).

A glance at the figures for passage V shows that on the first day (5 May 1938) division forms were present in the following percentages: 55 ($K+K$), 26 ($K+Ak$), 19 ($Ak+Ak$). On the next day (6 May) the percentage of non-dividing Ak individuals was found to have risen from 25 to 46 (at *a*). To account for this increase a theoretical estimate was made of the number of K and Ak trypanosomes which would result after n divisions, assuming that the individuals dividing irregularly (i.e. $K+Ak$) produce Ak and K forms in the ratio of $(2^n-1):1$ (*vide supra*, p. 535) and that Ak forms reproduce only Ak forms. The estimate was based on the following calculations:[1] the progeny of 100 dividing trypanosomes after n divisions will amount to (100×2^n); if α is the percentage of $K+K$ dividing forms, and β is the percentage of $K+Ak$ forms, the total number of K trypanosomes after n divisions will be $(2^n\,\alpha+\beta)$, and hence the number of Ak forms will be $(100\times 2^n)-(2^n\,\alpha+\beta)$. The number of divisions $(=n)$ for which these figures were calculated was determined by the nearest approximation of their ratio (cf. Table II, 5 May 1938, at *c*, *d*) to the ratio of K to Ak forms found on the following day (cf. Table II, 6 May 1938, at *a*). The calculations for the first day (5 May 1938) showed that after eight divisions there might be produced 14106 K forms and 11494 Ak forms, the ratio of which is as 54 to 44, i.e. very near to the actual ratio of the respective forms observed next day (viz. 54K to 46Ak: Table II, 6 May 1938, at *a*).

On subsequent days there was a gradual diminution in the number of Ak forms. Similar calculations made for each of these days again demonstrated that the estimated fall in the number of Ak forms after 8 divisions corresponded more or less closely to the fall actually observed on the next day (cf. Table II: 6 May, 7 May, 9 May, 10 May 1938 at *a*).

If we compare, in passage V (Table II), the percentage of K and Ak non-dividing forms on any day to the percentage of corresponding division forms on the same day, it will be seen that the percentage of divisions attributable to Ak forms ($Ak+Ak$: at *b*) was always disproportionately low as compared with that of divisions of K forms ($K+K$ and $K+Ak$). These figures would accordingly lead to expect a corresponding decrease in the number of Ak forms resulting from such divisions. However, in spite of the low percentage of $Ak+Ak$ divisions on 5 May 1938 there was a considerably higher proportion of Ak forms on 6 May 1938 (46 %) than on the preceding day (25 %). It is, therefore, evident that the fluctuation in this case was not influenced by the fall in the number of $Ak+Ak$ forms, and that some other factor was responsible.

As we have already shown, the other form of division affecting the number of Ak forms is the irregular division of K forms ($K+Ak$), which tends to produce Ak forms in progressively increasing numbers. An increase in these irregular divisions would result in a rise in the number of Ak forms, while a decrease would be followed by a fall in their number. The role of this factor is

[1] We are indebted to Dr J. C. Broom, of the Wellcome Bureau of Scientific Research, for suggesting the mathematical formulae which have considerably simplified the calculations.

clearly revealed if we correlate the variation in the percentage of $K+Ak$ dividing forms on successive days with the fluctuation in the proportion of Ak forms observed on the following days. Thus, on 5 May 1938 the percentage of $K+Ak$ forms reached 26 and next day (6 May 1938) the percentage of non-dividing Ak forms rose from 25 to 46. A lower percentage of $K+Ak$ forms (=17) on 6 May 1938 preceded a fall of the Ak forms from 46 to 34 % on 7 May 1938. A still further drop in the $K+Ak$ forms (=3 %) resulted in a diminution of Ak forms from 34 to 27 % on 9 May 1938, while the absence of any $K+Ak$ forms on the last date was followed, on 10 May 1938, by a fall in the number of Ak forms to 11 %.

It is not claimed that the foregoing calculations represent an exact mathematical analysis of the mechanism of fluctuation, but they undoubtedly lend support to our contention that the fluctuation in the proportion of Ak forms in this passage is due mainly to the number of irregular ($K+Ak$) divisions of the K forms. There can be no doubt, however, that other factors, such as a different rate of multiplication and/or a different death rate of the K and Ak forms also have an influence upon the relative numbers of these forms. To one of these factors may be due the low percentage of Ak forms on 10 May 1938 (=11), for in the absence of $K+Ak$ divisions the number of $Ak+Ak$ divisions (=21 %) on 9 May 1938 would lead to expect a higher percentage of Ak forms on 10 May 1938 than was actually observed (cf. also other figures at *a* and *b* in passage V: Table II).

The influence of these factors is more pronounced in passage XVIII *a* (Table II). Here we have a sudden rise in the percentage of Ak forms from 11 on 24 June 1938 to 30 next day, though the percentage of $K+Ak$ divisions is relatively low (=11), while that of $Ak+Ak$ divisions is disproportionately high (=30). In this case it would seem that the increase in akinetoplastic divisions contributed considerably to the rise in the proportion of Ak forms on the next day. The divisions occurring on 25 June 1938 point to an increase in the proportion of Ak forms on the following day (cf. Table II, 25 June 1938, at *d*), but unfortunately, in the absence of observations for 26 June 1938, this could not be verified. However, on 27 June 1938 the percentage of Ak forms remained on the same level. The divisions on this day suggest a slight increase of Ak forms (cf. Table II, 27 June 1938, at *d*) for the next day, but in fact there was a considerable fall in their numbers. This may have been due to increased mortality of the Ak forms, or to some other cause which is not clear.

From the foregoing observations it would appear that the fluctuation in the proportion of akinetoplastic trypanosomes in our strain depends upon the following factors, acting singly or in combination: (1) variation in the number of individuals dividing irregularly ($K+Ak$), which appears to be the most important factor; (2) variation in the rate of reproduction of the normal (producing $K+K$) and aberrant individuals (producing $Ak+Ak$); and (3) variation in the death rate of the different types of non-dividing and dividing forms.

It should be noted here that in an endeavour to determine the conditions

under which fluctuation takes place we have also considered the possible influence of the intensity of the infection upon the number of *Ak* forms at different times, but could find no correlation between them. In some cases a heavy infection coincided with a high percentage of these forms, and in others it was associated with a low percentage (=1); on the other hand, a very slight infection was sometimes associated with a low percentage and sometimes with a very high percentage (up to 50).

III. General considerations

The examples considered in the preceding section have provided some clues to the probable mechanism responsible for the fluctuation in the proportion of akinetoplastic forms in *T. evansi*. However, the *primary* cause of the disappearance of the kinetoplast and/or of the inhibition of its division, as well as the conditions affecting the fluctuation in the number of trypanosomes lacking this organ at different periods of the infection remain obscure, and at present no explanation can be advanced as to the cause of these phenomena.

It is possible, however, that the solution of the question lies in the elucidation of the nature and function of this structure, regarding which very little is known. In this connexion it is interesting to note that, according to Alexeieff (1917), the kinetoplast is an organ of mitochondrial origin, concerned with the production of glycogen, the presence of which was detected by microchemical reactions; in other words, it represents a plastid with a glycogenic function, i.e. a glycoplast, comparable to various plastids, such as leucoplasts, amyloplasts, chromoplasts, pyrenoids, stigmata, found in other flagellates and in plant cells. Lwoff & Lwoff (1931), though more guarded in their conclusions, have compared the kinetoplast to the stigma of Euglenoid flagellates, which is also a plastid. As is known, the plastids are cytoplasmic bodies of definite form, possessing the power of independent reproduction, each plastid being derived from a pre-existing one by bipartition. In all these particulars the kinetoplast of trypanosomes conforms to the plastids. Its similarity to these bodies is still more enhanced by the fact that in some flagellates, e.g. in the Chrysomonad *Rhizochrysis scherfeli*, "aplastic" (or "apoplastid") forms have been known to arise in the same way as akinetoplastic trypanosomes, viz. by failure of the plastid to divide (Lwoff, 1932).

Furthermore, according to Alexeieff (1917) the glycogen produced by the kinetoplast is deposited on its surface; if this is so, it is conceivable that its usual appearance and tinctorial reactions are attributable to the presence of glycogen and that the disappearance of this organ—whether induced or spontaneous—is due to a functional disturbance, as the result of which the kinetoplast ceases to produce glycogen and becomes invisible, either because of the minute size of the matrix or owing to its failure to stain by the usual methods employed. If this interpretation is correct the disappearance of the kinetoplast in trypanosomes is more apparent than real.

This hypothesis is admittedly speculative and is only tentatively advanced, in the hope that further investigations on the nature and function of the kinetoplast might provide a clue to the phenomena described in this paper. Some of the observed facts appear to support this hypothesis, while others do not.

In our previous paper (Hoare & Bennett, 1937, p. 55) it has been noted that in akinetoplastic strains a small percentage of trypanosomes appear to possess a kinetoplast varying in dimensions from full size to a minute dot in different individuals. Such forms have also been seen from time to time in the akinetoplastic strain described in the present paper. If the disappearance of the kinetoplast is due to the impairment of its function, it is possible that its normal function may be temporarily restored in certain individuals and this would account for the presence of trypanosomes with a kinetoplast in the aberrant strains. The very fluctuation in the proportion of akinetoplastic forms described in this paper is likewise suggestive of some functional disorder affecting the kinetoplast, but, on the other hand, it is difficult to apply this explanation to totally akinetoplastic strains of long duration, for it would imply that the impairment of function can be permanently established without in any way affecting all the other vital activities of the trypanosomes. Moreover, the appearance of normally constituted trypanosomes in akinetoplastic strains mentioned above can be explained in a more simple manner, for it is possible that the structures interpreted as kinetoplasts are actually artifacts, since various dark-staining granules frequently occurring in blood films may be indistinguishable from the kinetoplast when lying in the position which this organ usually occupies in the body of the trypanosome.

On the whole, it is clear that we are not yet in a position to offer a satisfactory explanation regarding the true nature of the phenomena discussed in this paper, and must leave the question open until more definite data are available.

IV. SUMMARY

An account is given of two aberrant strains of *Trypanosoma evansi* recovered from Sudanese camels.

One of these—like the four other ones described in a previous paper—was totally akinetoplastic (= akinetonucleate) and has been kept under observation for eighteen months in the course of which the aberrant condition remained unchanged.

The second strain was characterized by a wide fluctuation in the proportion of akinetoplastic forms which ranged from zero to over 70 % at different periods of observation.

The bearing of this fluctuation upon the production of akinetoplastic strains is discussed and it is concluded that these probably arise in nature either indirectly, through the inoculation by the insect vector of new hosts

with trypanosomes lacking the kinetoplast, or directly, by the strain becoming totally akinetoplastic within the same host.

From an analysis of the fluctuating strain it would appear that the variation in the proportion of akinetoplastic trypanosomes depends upon the following factors: (1) irregular division (without bipartition of the kinetoplast), which is the most important factor; (2) rate of reproduction of normal and aberrant individuals; (3) mortality rate of the different types of dividing and non-dividing forms.

The primary cause of the disappearance of the kinetoplast could not be ascertained.

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PURIFIED PREPARATIONS OF TOBACCO NECROSIS VIRUS (*NICOTIANA* VIRUS II)

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(With Plate XVII and 1 Text-figure)

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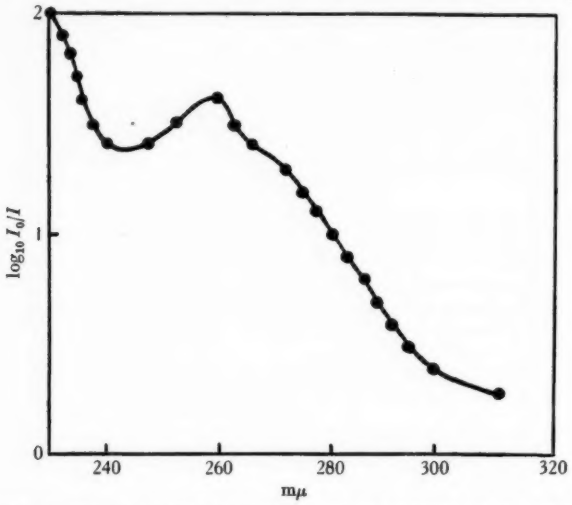
Introductory

Tobacco necrosis virus was discovered and described in 1935 (Smith & Bald). This name was chosen because the virus was first found in the tobacco plant, which is indeed the most usual host, and because the disease caused is only necrotic and lacks the leaf-mottling associated with many plant viruses. The virus is unusual in several respects, notably in its mode of dissemination (Smith, 1937*a*, *b*) and in the fact that it is normally confined to the roots of affected plants and does not become systemic in its host. It has only been detected in glasshouses. The only *natural* disease symptoms caused by this virus which have been observed occur occasionally in the two lowermost leaves of the tobacco plant and, still more rarely, of *Nicotiana glutinosa*. Nevertheless, the virus is frequently present in the roots of tobacco and many other plants without any visible indication of its presence. It is easily transmissible, by rubbing, to the leaves of tobacco on which it produces numerous necrotic spots which are sometimes in the form of rings (see Plate XVII, fig. 1). A certain amount of multiplication of virus takes place in the inoculated leaf, but there is no spread beyond this point and the disease is never systemic.

The fact that the virus never becomes systemic in the plant renders difficult its production in large quantities. Plants of various species were tested in the hope of finding one in which the virus would multiply systemically but without success, and the tobacco plant was finally used as the source of virus in these studies.

In order to obtain the maximum quantity of virus from the tobacco it is necessary to fulfil certain conditions. Young, rapidly growing tobacco plants, variety White Burley, were used and inoculated when they were about 10 in. across, the individual leaves being not greater than 4–5 in. in length. As a rule only three or four leaves out of each plant could be inoculated since lesions do not form either on the young rather sticky leaves or on older leaves if they exceed 6 inches in length. The addition of small quantities of carborundum powder



Text-fig. 1. Ultra-violet light absorption curve of a 0·013 % solution of tobacco necrosis virus preparation in a cell 2 cm. deep.

to the inoculum greatly increases the number of lesions produced. Under these conditions, and using as inoculum a sample of sap with a high virus content, the inoculated leaf should be completely covered with small circular lesions. It is important to collect these leaves at the right moment while they are still turgid since so many lesions cause the rapid collapse and death of the leaves. Between 800 and 1000 plants are necessary to produce 2 l. of sap with a high virus content, i.e. 0·04 %.

Purification of the virus

The chemical properties of the most active preparations of this virus that have been made closely resemble those of bushy stunt virus preparations. In spite of this, the method of isolation that proved satisfactory with bushy stunt (Bawden & Pirie, 1938*a*) does not give a good yield when applied to the sap of necrosis-infected tobacco plants. From among several methods that have been tried, only one need be given in detail, for it always gives good preparations.

The sap is expressed from minced leaves and mixed with half its volume of 97% alcohol. This precipitates the chlorophyll and starch, and also most of the

unstable normal proteins that would cause trouble later (Bawden & Pirie, 1938*b*), but it does not precipitate a serious amount of the virus. The precipitate separates immediately and can easily be centrifuged or filtered off. The clear pale brown fluid is mixed with two volumes of alcohol and left for an hour to precipitate. This precipitate also centrifuges easily, but it should be spun for half an hour at 3500 r.p.m. to get a tightly packed sediment, for alcohol interferes with the subsequent ammonium sulphate precipitation. The solid is suspended evenly in a volume of water equal to one-tenth of the original volume of the sap, and the insoluble material is centrifuged off and washed twice more with smaller quantities of water. The three extracts are mixed and half their volume of saturated ammonium sulphate solution is added; at first there is no precipitate, but in a few minutes an opalescence appears which flocculates in an hour. After three hours or more at room temperature the nearly white precipitate can be centrifuged off. There may be a few of the crystals characteristic of this virus preparation at this stage, but most of the crystalline material consists of calcium salts. The precipitate is suspended in a volume of water equal to 1/100 of the original volume of sap and centrifuged till nearly clear. If it contains the usual amount of virus it will necessarily be faintly opalescent. Judging by the serological titre, more than half of the virus is present in this fluid and further quantities can be isolated by washing the insoluble materials more thoroughly, or by adding more ammonium sulphate to the fluid from which virus has been precipitated.

This protein is more soluble in ammonium sulphate solutions at 0° C. than at room temperature; in this respect it resembles preparations of bushy stunt virus. Although it is known that a few other plant and animal proteins behave in this manner (Green, 1931) the property is sufficiently uncommon to serve as a valuable method of fractionation. Ammonium sulphate solution is added to the virus solution cautiously with stirring, till a precipitate just separates at 20° C. This happens when the fluid is about one-fifth saturated. The mixture is immediately cooled to 0° C. and if any solid remains undissolved, it is centrifuged off in a centrifuge kept at 0° C. The fluid is now kept in the ice chest, for, as with bushy stunt preparations, it is the amorphous material separating quickly which is more soluble at 0° C.; there is no marked difference in the solubility, at different temperatures, of the crystalline material that separates slowly. After a few hours a sheen develops in the fluid and after one or two days a precipitate which consists wholly or partly of thin lozenge-shaped plates separates. This is centrifuged off at 0° C. The supernatant fluid, on warming to room temperature, deposits an amorphous precipitate; from this more crystalline material can be prepared by repeating the process of adding ammonium sulphate solution till the fluid is turbid and then leaving the mixture in the ice chest. Crystallization will apparently proceed no further after the solution has lain at 0° C. for a week, but the supernatant fluid still contains two-thirds of the total virus activity. If more ammonium sulphate is added, a precipitate separates slowly at 0° C. but this, although it consists of

particles that are curiously regular in size, and although it gives a pronounced shimmer when agitated, is not crystalline. A further small amount of crystalline material can however be prepared from it by recrystallization. Many attempts have been made to get the remaining amorphous material into the crystalline state; these have been unsuccessful although part of a previously uncrystallizable preparation will often crystallize after "ageing" for some weeks at 0° C. Amorphous material does not crystallize when small amounts of the commonly occurring metal ions are added nor when it is added to tobacco sap and isolated over again. The virus may be recovered after prolonged incubation with trypsin and it can be sedimented by centrifuging for a few hours at 14,000 r.p.m. in a rotor of 8 cm. radius, but non-crystalline material remains non-crystalline after these attempts at purification.

The crystalline protein prepared by the method described is generally mixed with a little amorphous material. This mixture, like the similar mixture obtained in the early stages of a bushy stunt virus preparation, can be fractionated by washing it quickly with water. The pellet is mixed thoroughly with 5–10 times its bulk of water or dilute buffer solution at about *p*H 5 and centrifuged immediately. All the amorphous material but relatively little of the crystalline material dissolves under these conditions; the crystalline material dissolves completely only after several hours, the actual time depends on the amount of amorphous material present, for in its presence the crystalline material dissolves more rapidly. Material purified in this way may be recrystallized repeatedly without any alteration in its properties, and it may be crystallized in the same way after incubation with trypsin and after sedimentation by high-speed centrifugation. The crystals are stable in their mother liquor for many weeks. When dialysed and stored for a month there is little loss of activity and the material remains fully crystallizable; it is noticeable, however, that crystals made from old material dissolve in water even more slowly than any preparations of bushy stunt virus that have yet been handled. The greater part of a fresh virus preparation is amorphous, but after keeping at 0° C. for some weeks more crystalline material can generally be prepared from a dialysed solution by adding ammonium sulphate in the usual way. Attempts have been made to accelerate this "ageing" process by heating, freezing and exposure to dilute acid and alkali, but those attempts have so far been unsuccessful.

Neutral dialysed solutions of tobacco necrosis virus when dried in the frozen state give a light mass of open texture similar to that obtained with other virus preparations. This mass, like that from bushy stunt virus, but unlike that from tobacco mosaic virus, is insoluble in water. It is hygroscopic but not deliquescent and it has the following analytical composition:

C	44·8–45·3	H	6·5–7·0
N	15·5–16·5	P	1·4–1·7
S	1·1–2·00	Carbohydrate	7·0–9·0
	Ash	5·8–7·0	

The analyses were carried out by the methods used for other virus preparations (Bawden & Pirie, 1938*a*) and the figures for C, H, N and S fall within the usual range for proteins. The phosphorus and carbohydrate contents of these tobacco necrosis preparations are higher than those of the other plant virus preparations that have been studied. Nucleic acid of the ribose type has been isolated in crude form from these preparations after heat denaturation, but the small quantity of material available has precluded any quantitative work on the amount present. The strength of the ultra-violet absorption at 2600 A. is, however, compatible with the idea that, as in the other virus preparations, nucleic acid is responsible for the high phosphorus and carbohydrate content. The percentage of ash is, at first sight, unexpectedly high; the figure is derived from the weight of residue that is left after burning some neutral virus preparation in a current of oxygen for C and H determination. Since this protein has an isoelectric point in the neighbourhood of *p*H 4 part of this ash is due to the sodium hydroxide used for neutralization. Even after prolonged dialysis at *p*H 3 preparations contain 3–5 % of ash. This figure does not of course represent the actual metal content, for the ash contains, as would be expected, the greater part of the phosphorus of the nucleoprotein, presumably in the form of metaphosphate; the metal content is therefore only a fraction of the whole weight. The metal that is present in this virus preparation has not yet been studied.

Amorphous and crystalline materials have substantially the same analytical composition, and flocculate similarly with antisera, although crystalline preparations seldom flocculate beyond 1 : 320,000, whereas amorphous preparations often reach 1 : 640,000. The nature of the difference between them and the nature of the change which takes place when amorphous material "ages", giving rise to more crystalline material, is still not apparent to us. It seems unprofitable to frame any hypothesis to explain this change until more facts are available.

Material of each type has been studied on the ultra-centrifuge by Dr A. S. McFarlane. He finds no evidence for any inhomogeneity in solutions of the crystalline material and these sediment with a sharp boundary corresponding to $S_{W20°} = 130 \times 10^{-13}$. The amorphous material is inhomogeneous, but the principal component sediments with a diffuse boundary corresponding to $S_{W20°} = 58 \times 10^{-13}$. There are two or three other components in this mixture the heaviest of which has $S_{W20°} = 220 \times 10^{-13}$. Sedimentation constants of 130×10^{-13} and 58×10^{-13} would be given by spherical molecules of specific gravity 1·3 and molecular weights 7·4 and $2{\cdot}2 \times 10^6$. Dried preparations of tobacco necrosis have this specific gravity but it is not legitimate to assume that the component particles are spherical. Furthermore, if the particles in one type of preparation are spherical it is hard to see how the particles in the other type of preparation can be spherical also unless a deep-seated transformation takes place.

The crystals that separate from preparations containing much amorphous material are, as is found with other protein preparations, larger than those

obtained by the recrystallization of wholly crystalline material. The latter are seldom more than 10 μ across while the former may be 100 μ and it is this material that was photographed in Pl. XVII, figs. 2, 3. The presence of impurities does not apparently increase the thickness, and these large crystals are very fragile and are generally broken like one of those shown in Pl. XVII, fig. 2. On account of their thinness these crystals are not easy to photograph; the edges can however be made to stand out more clearly if some toluidine blue is added to this suspension after crystallization is complete. The crystals in Pl. XVII, fig. 3, were photographed in this way. When viewed flat these crystals do not show up between crossed nicols. In a hanging-drop preparation however many crystals are found lying at right angles to the water surface and therefore with their plane in the line of sight. These crystals, when examined edge-on in the polarizing microscope are birefringent and show sharp extinctions parallel to, and at right angles to, the plane of the crystal (Pl. XVII, fig. 4). Preparations of this virus, like those of bushy stunt, show no anisotropy of flow when in solution, and the jelly that sediments on high-speed centrifugation is also isotropic; physically the analogy between the two viruses cannot be carried further for bushy stunt crystallizes in the cubic system giving isotropic crystals whereas tobacco necrosis crystals are birefringent. It is perhaps justifiable to assume that the particles in a bushy stunt preparation are more or less spherical, but this assumption would not be justified by the form of tobacco necrosis crystals.

In an extensive study of the ultrafiltration of some twelve representative plant viruses it has been observed, judging solely by ease of filtration and consistency of filtration results, that these viruses fall into two classes. In one of these are the tomato bushy stunt and tobacco necrosis viruses while the remaining viruses form the other class. It is considered highly probable that the inconsistent filtration results without a sharp filtration end-point which have been obtained with these latter viruses are due to the fact that they are not spherical. On the other hand the available evidence suggests that the virus of tomato bushy stunt is a sphere and with this virus a sharp filtration end-point is invariably obtained, the virus passing regularly through all membranes down to that of the limiting pore size. While the filtration of tobacco necrosis virus does not approach this degree of consistency yet it exhibits a regularity of filtration which definitely ranges it with the spherical virus rather than with the other group of viruses which are thought to be rod-shaped.

Antibody production in rabbits

Injections of the purified tobacco necrosis preparation given intravenously into rabbits resulted in antibody formation.

A single intravenous dose of 0·1 mg. of the preparation was found to be effective in this respect, though more active antisera were produced as a result of a course of seven injections. The presence of antibody in the sera of animals so treated was demonstrated by flocculation reactions. Tests were

carried out in a total volume of 1·6 c.c. in 7 mm. test-tubes incubated at 40–45° C. in a glass-fronted water bath. Flocculation occurred in tubes containing antiserum dilutions up to 1 : 320 and antigen (virus preparation) to 1 : 320,000 invariably and often to 1 : 640,000. It was easily observable within the first half hour of incubation, increasing during the second half hour almost to its maximum.

Sera which flocculated the purified tobacco necrosis antigens gave no visible reaction with purified bushy stunt and tobacco mosaic antigens, nor with preparations of healthy sap made by subjecting the sap to the initial stages of the process used to purify the virus antigens. Preparations of the tobacco necrosis antigen gave no visible reaction with antisera prepared against the bushy stunt virus preparations.

The absence of cross reactions between these various reagents and the constancy with which flocculation occurred when tobacco necrosis antigen was mixed with its homologous antiserum make it highly probable that the antigen concerned is intimately associated with the tobacco necrosis virus if it is not that virus itself.

The floccules formed by the viruses of tobacco mosaic and Kenneth Smith's potato virus *X* with their respective homologous antisera are of a loose nebulous texture not unlike that of the floccules seen when bacterial flagella are agglutinated by their appropriate antibodies. The floccules of tobacco necrosis and its antiserum, however, are smaller and denser and on standing form a fairly compact deposit at the bottom of the tube. It is possible that the character of the floccules may be associated with the shape and size of the antigen particles.

The flocculation reaction has been used quantitatively to compare the flocculation powers of thirty different purifications or fractions of the virus-infected sap. These various antigen preparations have been titrated against one antiserum, varying the dilutions of antigen and keeping that of the antiserum constant at 1 : 100. The dilution end-points so obtained appear to be proportional to the amount of virus antigen in the preparation tested, though this has not been thoroughly checked for want of a sufficiently large batch of purified virus. The method, although quantitatively very rough, has served as a qualitative check on the efficacy of different purification processes.

INFECTIVITY TESTS

Although lesions are produced on the tobacco leaf in response to inoculation with tobacco necrosis virus, the sensitivity of response of this leaf to the virus is not great and this plant on the whole is not suitable as an accurate indicator of virus content. It has been shown previously (Smith, 1937*a*) that the French bean, *Phaseolus vulgaris*, is very much more sensitive to the virus and produces numerous small discrete lesions on the inoculated leaves which are

easily counted. The lesions must, however, be counted within a few days of their appearance since, as the virus moves rapidly along the vascular system, the lesions run together and lose their individuality.

The French bean was used throughout as a test plant and inoculations were made by means of a glass spatula with a ground-glass face. The spatula was dipped once into the virus suspension for each half-leaf inoculated. The results of some comparative dilution tests with purified virus preparations and crude virus sap are given in Table I. Each inoculation was made to four leaves of French bean.

Table I. *Activity of purified preparations of tobacco necrosis virus compared with that of crude clarified virus sap*

	Infectivity on *Phaseolus vulgaris* var. Canadian Wonder Average number of lesions per leaf at various dilutions					
Virus sample	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Purified virus, sample 1	—	—	119	36	9	—
Purified virus, sample 2	—	394	148	31	—	—
Purified virus, sample 3	—	357	36	8	—	—
Purified virus, sample 4	—	—	476	143	36	—
Purified virus, sample 5	—	—	325	117	60	15
Crude clarified virus sap, sample 1	4	0	0	—	—	—
Crude clarified virus sap, sample 2	6	0	0	—	—	—
Crude clarified virus sap, sample 3	12	2	0	—	—	—

Effect of freezing

In many ways these virus preparations resemble those of bushy stunt virus; neither shows anisotropy of flow, there is no *p*H value in the range of stability at which they are insoluble in water and they are more soluble in ammonium sulphate solution at 0° C. than at room temperature. When frozen, however, they differ in their behaviour. Bushy stunt preparations become quite insoluble and inactive whereas tobacco necrosis preparations retain their solubility in water even after repeated freezing and thawing, and the solution loses to a great extent the opalescence characteristic of virus preparations. The change which takes place on freezing has not been analysed in detail but it is clear that it leads to a fall in infectivity and in precipitability with antisera especially in regions of antigen excess.

Summary

Two nucleoproteins with similar chemical composition have been isolated from the leaves of tobacco plants infected with tobacco necrosis virus. One of

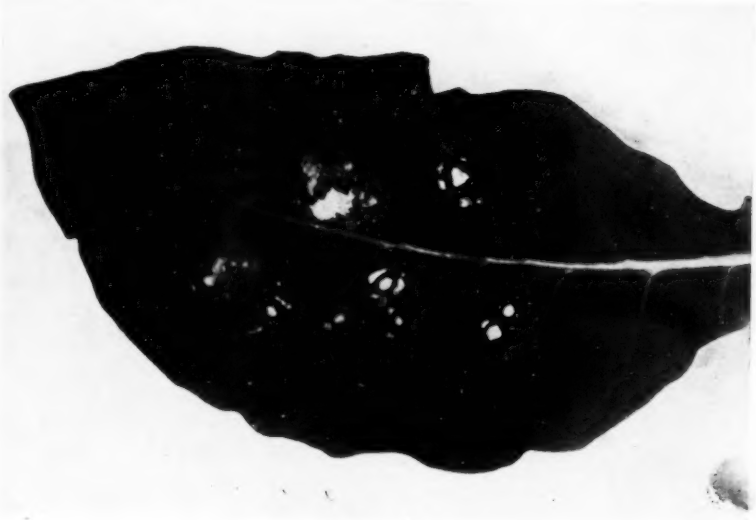


Fig. 1.

Fig. 2.

Fig. 3.

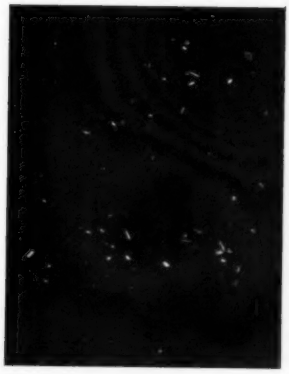


Fig. 4.

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these is crystalline and has a sedimentation constant of 130×10^{-13}; the other is amorphous and its principal component has a sedimentation constant of 58×10^{-13}.

Each preparation will infect plants at a dilution of 1 in 10^8 and will precipitate specifically with antiserum at a dilution of 1 in $3{\cdot}2 \times 10^5$.

The nature of the difference between preparations in the two states is obscure and it has not proved possible to convert the one into the other.

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EXPLANATION OF PLATE XVII

Fig. 1. Leaf of tobacco plant, var. White Burley, experimentally infected with the virus of tobacco necrosis. $\frac{1}{2}$ natural size.
Fig. 2. Photomicrograph of crystals from a purified preparation of tobacco necrosis virus. × 100.
Fig. 3. Photomicrograph of crystals stained with toluidine blue. × 100.
Fig. 4. Photomicrograph of crystals in polarized light. × 100.

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